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GENETICS OF SYSTEMIC AUTOIMMUNITY AND COMORBIDITIES

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Genetics of systemic autoimmunity and comorbidities

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By

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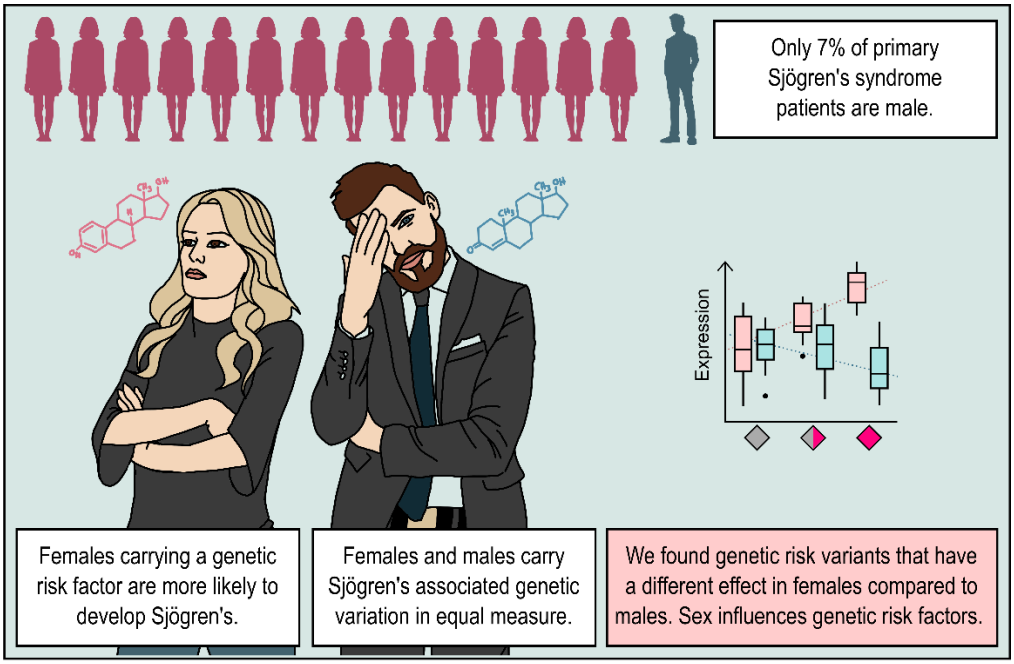

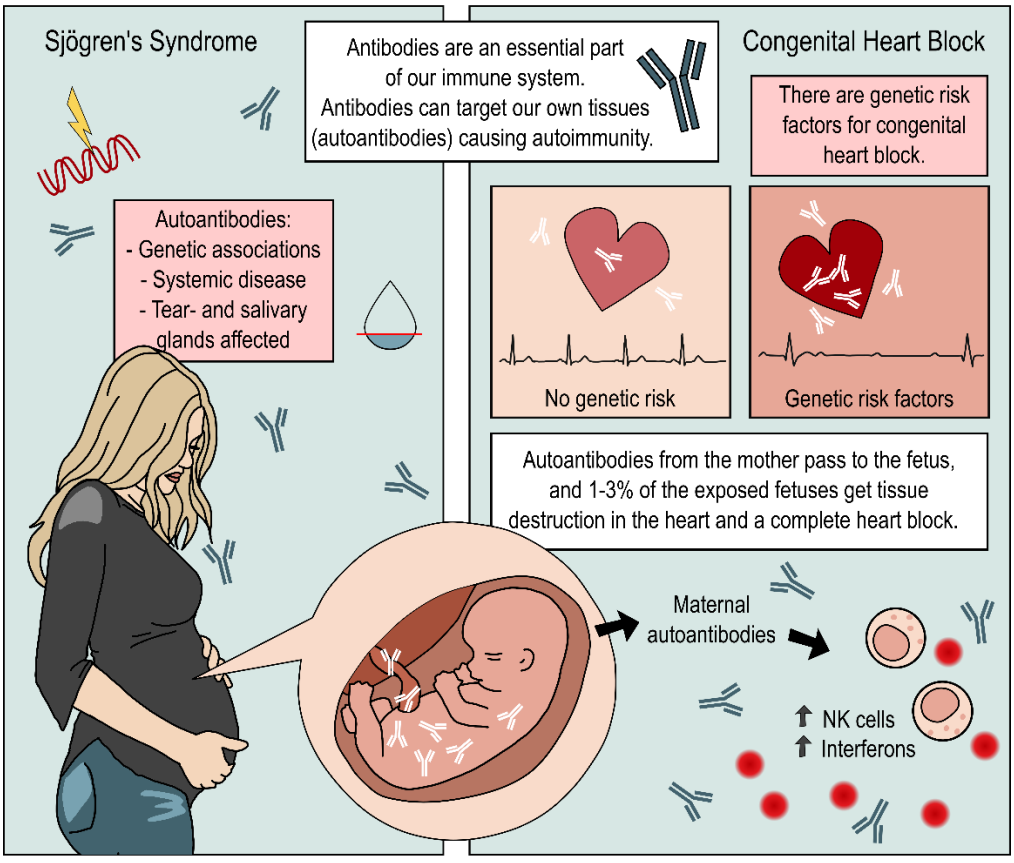

ABSTRACT

Sjögren‘s syndrome (SS) is a systemic autoimmune disease that typically affects the salivary and lacrimal glands leading to dry eyes and dry mouth, but the patients may also have systemic involvement. Most patients with SS carry autoantibodies, most often to the Ro (SSA) and La (SSB) proteins. During pregnancy, these autoantibodies can pass over from affected mothers to the fetus, who is then at risk of developing a congenital heart block (CHB). There is a prominent gender bias in systemic autoimmunity overall, particularly in SS where the ratio of women to men is approximately 14:1. While some genetic susceptibility factors for SS have been identified, much about the interplay between genetics, sex and the varying clinical presentation of this disease needs further study.

In order to identify genetic associations with SS, we performed targeted sequencing of 1853 immune-related loci in over a thousand clinically well characterized SS patients and population controls. After subgrouping the patients by clinical manifestations, we identified three independent genetic signals in the *HLA* locus for Ro/La autoantibody positive SS, that were absent in the antibody negative group. The two top associations further indicated higher risk of severe manifestations, including purpura, major salivary gland swelling and lymphadenopathy. Our findings demonstrate that two distinct subgroups of patients with SS can be defined by the presence or absence of Ro/La antibodies and genetic markers.

We and others have identified genetic variants associated with SS and/or the partially overlapping disease systemic lupus erythematosus (SLE). The frequency of these variants is the same in women and men in the general population. However, as the vast majority of patients are women, the risk of developing SS or SLE is much higher if the carrier is female. Genetic variants may contribute to different phenotypes via differential gene regulation, so called expression quantitative trait loci (eQTL). We hypothesized that sex may influence the eQTL effects of SS/SLE-associated genetic variants differentially in women and men, and discovered several associated variants that had sex-specific effects on the expression of nearby genes in CD19+ B cells. We also specifically studied the SS/SLE-associated *FAM167A-BLK* locus on chromosome 8, since the associated variants in this locus have prominent eQTL effects on the unknown gene *FAM167A*. We found the gene to be evolutionarily conserved, with one additional family member, but no other homologies in the genome, and the proteins to have a high level of intrinsic disorder. The gene is expressed in B cells, and we further found high expression in the lung of both mice and humans. We named the *FAM167* encoded proteins Disordered Autoimmunity 1 and 2 (DIORA1 and 2), and will continue our efforts to further characterize their function.

CHB affects 1-3% of anti-Ro/La autoantibody exposed pregnancies, with a recurrence rate of approximately 12-16%. The relatively low recurrence rate, despite persistent maternal autoantibodies, suggests fetal susceptibility factors are important. We performed a genome-wide association study in patients with CHB and controls. We found genetic associations with variants in the *HLA* region, *KCNT2* on chromosome 1, and several suggestive signals. Most women with Ro/La autoantibodies have a steady state interferon activation, and to study the interferon in anti-Ro/La autoantibody positive pregnancy, we sampled anti-Ro/La positive mothers and their neonates at the time of birth, as well as healthy control mother-child pairs, and analyzed cellular profiles and cytokine- and gene expression. We found that anti-Ro/La exposed neonates had measurable type I and II interferon in plasma, and increased NK cell frequencies. Intracellular interferon detected by flow cytometry in neonatal NK and T cells indicates the neonates may produce the interferon, and we confirmed neonatal cell ability for interferon production *in vitro*. Notably, the genes near the CHB-associated signals were enriched for interferon regulation, suggesting an interplay between interferon activation and fetal genetic susceptibility factors in the pathogenesis of CHB.

In summary, this thesis presents novel insight into genetic associations with SS and CHB, and begins to delineate how genetic susceptibility interacts with biological context such as sex or intrauterine exposure to maternal immune factors to lead to disease. The results will be important in clinical practice for personalized treatment and follow-up strategies, and as a basis for future therapy development.

LIST OF SCIENTIFIC PAPERS

I. **Genetic and clinical basis for two distinct subtypes of primary Sjögren's syndrome.**
Guðný Ella Thorlacius, Lina Hultin-Rosenberg, Johanna K Sandling, Matteo Bianchi, Juliana Imgenberg-Kreuz, Pascal Pucholt, Elke Theander, Marika Kvarnström, Helena Forsblad-d'Elia, Sara Magnusson Bucher, Katrine B Norheim, Svein Joar Auglænd Johnsen, Daniel Hammenfors, Kathrine Skarstein, Malin V Jonsson, Eva Baecklund, Lara A Aqrawi, Janicke Liaaen Jensen, Øyvind Palm, Andrew P Morris, DISSECT consortium; the ImmunoArray consortium; Jennifer R S Meadows, Solbritt Rantapää-Dahlqvist, Thomas Mandl, Per Eriksson, Lars Lind, Roald Omdal, Roland Jonsson, Kerstin Lindblad-Toh, Lars Rönnblom, Marie Wahren-Herlenius, Gunnel Nordmark
Rheumatology (Oxford). 2020 Aug 21;keaa367.

II. **Sex influences eQTL effects of SLE and Sjögren's syndrome-associated genetic polymorphisms.**
Magdalena Lindén, Jorge Ivan Ramírez Sepúlveda, Tojo James, Guðný Ella Thorlacius, Susanna Brauner, David Gómez-Cabrero, Tomas Olsson, Ingrid Kockum, Marie Wahren-Herlenius.
Biol Sex Differ. 2017 Oct 25;8(1):34.

III. **The rheumatic disease-associated FAM167A-BLK locus encodes DIORA-1, a novel disordered protein expressed highly in bronchial epithelium and alveolar macrophages**.
Lara Mentlein, Guðný Ella Thorlacius, Lauro Meneghel, Lara Adnan Aqrawi, Jorge Ivan Ramírez Sepúlveda, Johan Grunewald, Alexander Espinosa, Marie Wahren-Herlenius.
Clin Exp Immunol. 2018 Aug;193(2):167-177.

IV. **Genome-wide association study of autoimmune-mediated congenital heart block in Europeans reveals association with variants in the *HLA* region and *KCNT2***.
Guðný Ella Thorlacius, Klementy Shchetynsky, Lauro Meneghel, Malin Hedlund, Vijole Ottosson, Alexander Espinosa, Kalliopi Kazamia, Heikki Julkunen, Marianne Eronen, Ariela Hoxha, Cathrine Ebbing, Sabrina Meisgen, Stina Salomonsson, Joanna Tingström, Margarita Ivanchenko, The Swedish Congenital Heart Block Study Group, Kristina Gemzell-Danielsson, Jan Hillert, Lars Alfredsson, Pernilla Stridh, Tomas Olsson, Sara De Carolis, Torvid Kiserud, Amelia Ruffati, Athanasios G Tziofas, Andreas Früh, Juha Kere, Gunnar Bergman, Håkan Eliasson, Sven-Erik Sonesson, Ingrid Kockum, Marie Wahren-Herlenius
Manuscript

V. **Type I IFN system activation in newborns exposed to Ro/SSA and La/SSB autoantibodies *in utero*.**
Malin Hedlund*, Guðný Ella Thorlacius*, Margarita Ivanchenko, Vijole Ottosson, Nikolaos Kyriakidis, Linda Lagnefeldt, Joanna Tingström, Allan Sirsjö, Anders A Bengtsson, Emma Aronsson, Kristina Gemzell-Danielsson, Lars Ronnblom, Gunnar Bergman, Alexander Espinosa, Sven-Erik Sonesson, Maija-Leena Eloranta, Marie Wahren-Herlenius
RMD Open . 2020 Jan;6(1):e000989.

VI. **Natural killer cells and type II interferon in Ro/SSA and La/SSB autoantibody-exposed newborns at risk of congenital heart block**.
Margarita Ivanchenko, Guðný Ella Thorlacius, Malin Hedlund, Vijole Ottosson, Lauro Meneghel, Sophia Björkander, Amina Ossoinak, Joanna Tingström, Katarina Bremme, Eva Sverremark-Ekström, Kristina Gemzell-Danielsson, Sven-Erik Sonesson, Karine Chemin*, Marie Wahren-Herlenius*
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*Equal contribution

LIST OF SCIENTIFIC PAPERS NOT INCLUDED IN THE THESIS

Augmented Th17 differentiation in Trim21 deficiency promotes a stable phenotype of atherosclerotic plaques with high collagen content.
Susanna Brauner, Xintong Jiang, Guðný Ella Thorlacius, Anna M Lundberg, Therese Östberg, Zhong-Qun Yan, Vijay K Kuchroo, Göran K Hansson, Marie Wahren-Herlenius
Cardiovasc Res. 2018 Jan 1;114(1):158-167.

An update on the role of type I interferons in systemic lupus erythematosus and Sjögren's syndrome.
Guðný Ella Thorlacius, Marie Wahren-Herlenius, Lars Rönnblom
Curr Opin Rheumatol. 2018 Sep;30(5):471-481.

The Sjögren's syndrome-associated autoantigen Ro52/TRIM21 modulates follicular B cell homeostasis and immunoglobulin production.
Susanna Brauner, Margarita Ivanchenko, Guðný Ella Thorlacius, Aurélie Ambrosi, Marie Wahren-Herlenius
Clin Exp Immunol. 2018 Dec;194(3):315-326.

Clinical associations and expression pattern of the autoimmunity susceptibility factor DIORA-1 in patients with primary Sjögren's syndrome.
Lara A Aqrawi*, Lara Mentlein*, Lauro Meneghel, Albin Björk, Guðný Ella Thorlacius, Margarita Ivanchenko, Jorge I Ramírez Sepúlveda, Kathrine Skarstein, Marika Kvarnström, Susanna Brauner, Alexander Espinosa, Marie Wahren-Herlenius
Ann Rheum Dis. 2018 Dec;77(12):1840-1842.

Protein and DNA methylation-based scores as surrogate markers for interferon system activation in patients with primary Sjögren's syndrome.
Albin Björk, Elina Richardsdotter Andersson, Juliana Imgenberg-Kreuz, Guðný Ella Thorlacius, Johannes Mofors, Ann-Christine Syvänen, Marika Kvarnström, Gunnel Nordmark, Marie Wahren-Herlenius
RMD Open. 2020 Jan;6(1):e000995.

Viral antigens elicit augmented immune responses in primary Sjögren's syndrome.
Albin Björk, Guðný Ella Thorlacius, Johannes Mofors, Elina Richardsdotter Andersson, Margarita Ivanchenko, Joanna Tingström, Tojo James, Karl A Brokstad, Rebecca J Cox, Roland Jonsson, Marika Kvarnström, Marie Wahren-Herlenius
Rheumatology (Oxford). 2020 Jul 1;59(7):1651-1661.

CONTENTS

LIST OF ABBREVIATIONS

ABHD6	Abhydrolase Domain Containing 6, Acylglycerol Lipase
ACA	Anti-centromere antibody
ANA	Anti-nuclear-antibody
ARCN1	Archain 1
AV	Atrioventricular
BAFF	B-cell activating factor
BANK1	B Cell Scaffold Protein With Ankyrin Repeats 1
BLK	B lymphoid kinase
C2	Complement C2
C3	Complement C3
C4A	Complement C4A (Rodgers Blood Group)
CD	Cluster of differentiation
CFH	Complement Factor H
CFHR3	Complement Factor H Related 3
CHB	Congenital heart block
CHiCP	Capture HiC Plotter
Chr	Chromosome
COVID-19	Coronavrus Disease 2019
CTSB	Cathepsin B
DELFIA	Dissociation-enhanced lanthanide fluorescence immunoassay
DHX9	DExH-Box-Helicase 9
DIORA	Disordered autoimmunity
DNA	Deoxyribonucleic acid
DNASE1L3	Deoxyribonuclease 1 Like 3
DUF3259	Domain of unknown function 3259
ELISA	Enzyme-linked immunosorbent assay
eQTL	Expression quantitative loci
FAM167	Family with sequence similarity
GO	Gene Ontology
GOT1	Glutamic-oxaloacetic transaminase 1
GRM	Genetic relationship matrix
GTEx	Genotype-Tissue Expression project
GWAS	Genome-wide association study
HCP5	HLA Complex P5
HLA	Human Leukocyte Antigen
IFI44	Interferon induced protein 44
IFIT1	Interferon Induced Protein With Tetratricopeptide Repeats 1
IFN	Interferon
IgG	Gamma immunoglobulin
IRF	Interferon regulatory factor
ISG15	ISG15 Ubiquitin Like Modifier
LY6E	Lymphocyte Antigen 6 Family Member E
MAP2K2	Mitogen-Activated Protein Kinase Kinase 2
meQTL	Methylation quantitative trait loci
MHC	Major histocompatibility complex
miR	micro-RNA

MX1/2	MX Dynamin Like GTPase 1/2
NCF2	Neutrophil Cytosolic Factor 2
NK	Natural Killer
OAS1/2	2'-5'-Oligoadenylate Synthetase 1/2
OASL	2'-5'-Oligoadenylate Synthetase Like
PBMC	Peripheral blood mononuclear cell
PDHB	Pyruvate Dehydrogenase E1 Subunit Beta
PLSCR1	Phospholipid Scramblase 1
PXK	PX Domain containing Serine/Threonine Kinase like
RF	Rheumatoid factor
RNA	Ribonucleic acid
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIGLEC	Sialic acid-binding immunoglobulin (Ig)-like lectins
SIMOA	Single molecule array
SLC39A8	Solute Carrier Family 39 Member 8
SLE	Systemic Lupus Erythematosus
SNP	Single nucleotide polymorphism
Src	SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase
SS	Sjögren's Syndrome
SSA	Sjögren's-syndrome-related antigen A
SSB	Sjögren's-syndrome-related antigen B / Small RNA Binding Exonuclease Protection Factor La
STAT1	Signal Transducer And Activator Of Transcription 1
TAP1/2	Transporter 1/2, ATP Binding Cassette Subfamily B Member
TAPBP	TAP Binding Protein
TDT	Transmission disequilibrium test
TLR	Toll like receptor
TNIP1	TNFAIP3 Interacting Protein 1
TREX	Three-Prime Repair Exonuclease
UCSC	University of California, Santa Cruz
UHRF1BP1	UHRF1 Binding Protein 1
XIST	X Inactive Specific Transcript

1 INTRODUCTION

Autoimmunity is an umbrella term for any disorder caused by the immune system actively attacking any system in the body, or failing to temper immune processes initiated by outside sources, often leading to tissue destruction and/or a persistent autoimmune disease state. With the emergence of biologics - potent targeted therapies based on inhibiting particular processes within the immune system - many autoimmune diseases have seen a drastic improvement in the variety and efficacy of the available treatment options.[2] However, preventive or curative treatment has not been found for most of these diseases, so further study is still needed.

1.1 PRIMARY SJÖGREN'S SYNDROME

Primary Sjögren's syndrome is a systemic autoimmune disease characteristically affecting exocrine glands, particularly the lacrimal and salivary glands, leading to dryness of mucosal surfaces, most prominently of the eyes and mouth. Secondary Sjögren's syndrome can be diagnosed when an individual with an established autoimmune disease develops symptoms of Sjögren's syndrome. No patients with secondary Sjögren's syndrome have been included in the studies in this thesis, thus, only primary Sjögren's syndrome will be discussed and referred to only as Sjögren's syndrome hereafter.

The incidence rate of Sjögren's syndrome is around 3 per hundred thousand adults, and the prevalence around 0.05-0.1%.[3] The majority of patients with Sjögren's syndrome are females over 40 years old (males make up less than 7% of the patients).[4-6] The most common symptoms are dryness of the eyes and mouth, but many will develop extraglandular manifestations such as Raynaud's phenomenon or purpura. A systemic activation of interferon responses is also a common manifestation, with more than two thirds of patients showing signs of interferon activity.[7,8] Anti-nuclear antibodies, anti-Ro/SSA and/or anti-La/SSB autoantibodies are present in the majority of the patients, as well as other autoantibodies such as rheumatoid factor. Sjögren's syndrome autoantibodies will be discussed in more detail below. The presence of the anti-Ro/SSA and/or anti-La/SSB (hereafter referred to as anti-Ro/La) autoantibodies also confers a risk of neonatal lupus syndrome developing in children born to autoantibody positive mothers, also discussed in more detail below.

Patients with Sjögren's syndrome have an increased mortality due to an increased risk of lymphoma,[9,10,11] and for a subset of patients, their Sjögren's syndrome diagnosis can even be concomitant with a lymphoma diagnosis, particularly in men.[12] These lymphomas are most often, but not exclusively, diffuse B cell lymphomas or marginal zone lymphomas,[13] but an additional risk of non-Hodgkin's lymphoma comes with germinal-center like structures in infiltrated minor salivary glands.[14]

Great strides have been taken in the treatment of many autoimmune diseases since the turn of the last century. However, several clinical trials of varied sizes have tested the safety

and efficacy of various biological treatments for Sjögren's syndrome, but most have failed to meet their primary endpoints (reviewed in:[15]). These trials included tests of Abatacept,[16] and Rituximab (anti-CD20 treatment to deplete B cells) which showed moderate improvement of clinical outcomes in early trials,[17,18] but was found to not be sufficiently effective in later trials.[19] B-cell activating factor (BAFF) or BAFF receptor blockade (belimumab or ianalumab, respectively), also had a promising start,[20,21] but still no breakthrough in later trials.[22] The lack of success seen in some of these trials revealed a need for patient stratification before inclusion in clinical trials, and more studies on disease sub-phenotypes for Sjögren's syndrome.

There is a consensus in the field that some differentiation between patient subgroups is necessary. In 2015, Seror *et al.*[23] found that there was a subgroup of patients, identified as having a prominent systemic type I interferon activation, which responded better to belimumab than their interferon-negative counterparts. More recently, age at onset has been suggested as a useful marker for distinguishing subgroups of Sjögren's syndrome patients more likely to have more severe forms of disease.[24] These studies match others stating several factors as potential defining features for subgrouping patients with Sjögren's syndrome. In fact, a great number of parameters can vary from one patient to another and several methods have been tested to find the most relevant grouping variables, including the use of artificial neural networks.[25] Below, I will discuss some of the prominent features of Sjögren's syndrome and related diseases in more detail.

1.1.1 Autoantibodies

It is common for patients diagnosed with Sjögren's syndrome to carry autoantibodies. The characteristic autoantibodies found in patients with Sjögren's syndrome are the anti-Sjögren's syndrome-related antigen A or B (anti-SSA/Ro or anti-SSB/La) autoantibodies targeting Ro52 (*TRIM21*), Ro60 (*TROVE2*) or La (*SSB*). These autoantibodies can be present up to two decades before diagnosis of Sjögren's syndrome,[26] and around 75% of patients with Sjögren's syndrome are anti-Ro positive.[27] Anti-Ro autoantibodies are also included in the most recent classification criteria for Sjögren's syndrome by the 2016 American College of Rheumatology and European League Against Rheumatism,[28] making these antibodies a defining feature of Sjögren's syndrome and an important diagnostic marker.

The Ro/La autoantibodies can be found in combination with each other, other autoantibodies (most notably other anti-nuclear-antibodies, ANAs), or solitary. Looking at only the anti-Ro positive, reports have showed isolated anti-Ro52 as being the most common (43% in: [29]), and the same report found a third of anti-Ro/La carriers had both anti-Ro52 and anti-Ro60 autoantibodies, and a quarter was found to carry anti-Ro60 only. They also found that anti-Ro52 and Ro60 double-positivity correlated with Sjögren's syndrome diagnosis, while isolated Ro52 or Ro60 correlated better with other autoimmune diagnosis. While anti-La autoantibodies are most common in carriers of anti-Ro, a fraction of patients can be found to carry only anti-La. The phenotype of isolated anti-La, however, is a matter of some debate. Clinical features of isolated anti-La individuals have been suggested to not differ significantly

from antibody-negative individuals,[30] but systemic associations with isolated anti-La have also been described.[31] Overall, Sjögren's syndrome patients carrying anti-Ro and/or La autoantibodies are more likely to have lymphocytic infiltrations of affected glands, systemic manifestations, B cell expansion and risk of lymphoma than their anti-Ro/La negative counterparts.[5,32,33]

In the absence of anti-Ro/La autoantibodies, several alternatives have been suggested as prognostic markers for Sjögren's syndrome, including ANAs, rheumatoid factor (RF) and anti-α-fodrin antibodies.[34] Most of the different autoantibodies associate with different clinical presentations of Sjögren's syndrome.[35] ANAs without anti-Ro/La have been found to correlate specifically with primary biliary cirrhosis in Sjögren's syndrome patients, and in combination with anti-Ro specifically with polyneuropathy,[36] but co-occurrence of ANA and anti-Ro/La is a common feature in Sjögren's syndrome. Cryoglobulins - immunoglobulins that precipitate at temperatures below 37°C - have been proposed as a prognostic marker for overall mortality[9] and lymphoma development,[37] specifically cryoglobulinemic vasculitis[38] in patients with Sjögren's syndrome. Anti-centromere antibodies (ACAs) rarely overlap with anti-Ro/La, but have been suggested to define a distinct subgroup bordering both limited scleroderma and Sjögren's syndrome with a different disease course but high risk of non-Hodgkin's lymphoma.[39,40] Patients with dry eyes or mouth who have hypergammaglobulinemia without enough other diagnostic markers to get a diagnosis of Sjögren's syndrome are more likely to progress towards a diagnosis in a couple of years than those who lack both hypergammaglobulinemia and hypocomplement.[41] So overall, not only the presence of particular autoantibodies but also hypergammaglobulinemia (increased levels of polyclonal gamma immunoglobulins (IgG)) can also be a marker for increased risk of Sjögren's syndrome.

1.1.2 Genetics

Over the course of the last two decades, genetics have been shown to be an important risk factor for the development of autoimmunity. Having a first degree relative with Sjögren's syndrome makes one more likely to develop Sjögren's syndrome, or another autoimmune disease,[42] so there is a clear genetic component to disease susceptibility. Several studies have found associations between genetic variants, particularly variation in the *HLA* locus on chromosome 6, with an overall risk for Sjögren's syndrome development. Below, I will broadly summarize the main findings made in this field so far.

The result of the first genome-wide association study of Sjögren's syndrome in Caucasians was published in 2013,[43] which confirmed previously identified genetic associations, mainly with the *HLA* region, and revealed new associations. The loci/regions most significantly associated, after *HLA*, were the *IRF5-TNPO3, STAT4, IL-12A, FAM167A-BLK, DDX6-CXCR5* and *TNIP1* loci, and a number of suggestive associations were also described.

Associations with *IRF5* and *STAT4*[44], *BLK-FAM167A, TNFSF4* and *EBF1,*[45] had previously been published in candidate-gene studies in the Scandinavian population, and a candidate-gene study published at a similar time in 2013 confirmed the association with *TNIP1* variants in Scandinavians,[46] particularly in anti-Ro positive patients. Many of these associations have been replicated in samples from patients of other ethnicities,[47,48] and several associations have been identified since (reviewed in: [49,50]). Among them, *GTF2I* has been found to be associated with Sjögren's syndrome in Han Chinese but not Caucasians,[48,51,52] and contradicting findings have been made in regards to associations with *TNFAIP3*.[53,54] Overall, there is a heterogeneity in both the clinical presentation and the genetic associations found with Sjögren's syndrome depending on ancestry,[55] and studies on more detailed subgroup analysis might yield new insight into disease pathogenesis.

Like *HLA, STAT4* and *IRF5,* the *BLK-FAM167A* locus on chromosome 8 has been associated with other autoimmune diseases, including: rheumatoid arthritis [56], systemic lupus erythematosus,[57] systemic sclerosis,[58] dermatomyositis,[59] primary antiphospholipid syndrome,[60] and finally Kawasaki disease, where the association with the *BLK-FAM167A* locus was the most significant finding.[61] However, when looking into expression quantitative trait loci or eQTL effects of the associated variants, the *BLK-FAM167A* locus stands out, because of the prominent effect of the associated genotype on the expression of *FAM167A* in B cells.[62] The *Family with sequence similarity 167* or *FAM167* gene family contains two members*; FAM167A* (*C8orf13*) on chromosome 8p23.1 and *FAM167B* (C*1orf90*) on chromosome 1p35.1. The genes have been grouped by shared sequence similarity but share no homology to other known genes or domains, so what role *FAM167A* might play in the pathogenesis of autoimmune diseases is unknown.

The associations mentioned above vary in their distribution and effects, and much is yet to be discovered in this field, but the strongest genetic association in studies on any population of Sjögren's syndrome patients tested so far remain with variants in the *HLA* region. For decades, we have been aware of an association between Sjögren's syndrome and *HLA* variants.[63,64] In a meta-analysis covering 1166 cases with Sjögren's syndrome and 6470 controls published in 2012, the association with *HLA* class *II* genes was mapped to DQA1*0501, DQB1*0201 and DRB1*0301,[65] a finding confirmed by Lessard *et al*., in 2013,[43] well establishing that particular *HLA* alleles confer a risk of developing Sjögren's syndrome.

In addition to genetic variation, recent evidence has also pointed towards a role for epigenetic regulation in disease pathogenesis. Numerous Sjögren's syndrome associated genetic variants have been shown to have methylation quantitative trait loci (meQTLs).[66-68] Interestingly, interferon regulated genes are hypomethylated in whole-blood, B cells and minor salivary gland biopsies from patients with primary Sjögren's syndrome,[69] supporting a role for epigenetics in the induction and/or maintenance of interferon responses found to be aberrant in patients with Sjögren's syndrome (discussed further below). Epigenetics and genetic associations with Sjögren's syndrome are reviewed in:[50].

1.1.3 Sex

Males make up less than a tenth of all Sjögren's syndrome patients, yet male sex is a risk factor for increased mortality and/or more severe systemic disease.[9,70,71] We know that being female confers a greater risk of developing autoantibodies and autoimmune disorders such as Sjögren's syndrome, but we don't know why.

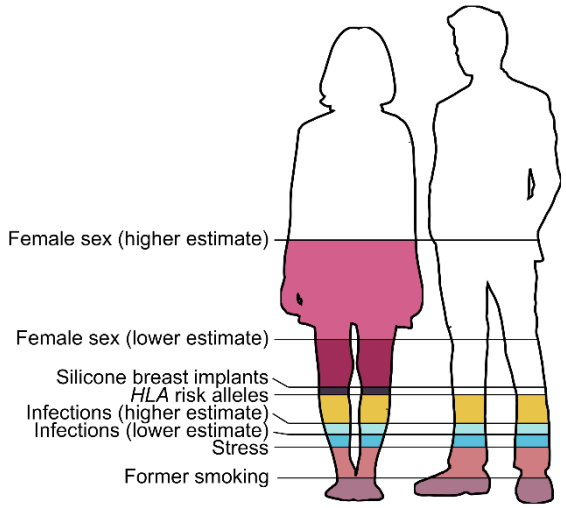

Figure 1: Comparison of sex as a risk factor for primary Sjögren's syndrome compared to examples of other risk factor estimates as summarized in [1].

The female to male ratio in Sjögren's syndrome is not a static variable, but one very dependent on geolocation,[27] ranging from as low as 7:1 in black/African-American populations, to 27:1 in Asian patients (Figure 1). The same can be said for the frequency of associated genetic variants, which can differ drastically between populations,[55,72] as well as the clinical presentation of the disease,[73] so filtering out the signal from the noise in the hunt for risk factors for Sjögren's syndrome can prove difficult. However, given the amount of time, money and effort that has been spent on defining both genetic and environmental risk factors for sex-influenced autoimmune disorders, the influence of sex remains remarkably understudied.

Sex hormones have long been suspected to drive the sexual dimorphism seen in many autoimmune disease, and with good reason. Examples of systemic lupus erythematosus onset after male to female gender reassignment suggest a direct connection with sex hormones,[74] and the onset of diseases like Sjögren's syndrome being concomitant with drops in both estrogen, as well as androstenedione and testosterone levels in women after menopause[75] make a compelling argument for a role for sex hormones in disease development, however, the presence of autoantibodies decades before disease onset would suggest that additional risk factors are needed. One of the proposed mechanisms for sex hormone influence on autoimmunity is androgen-induced immunosuppression (reviewed in: [76]). Androgen receptors are widely expressed, and variations in androgen levels have been associated with fluctuations in immune function.[77] Estrogens can also modify the potency of immune responses in a myriad of ways, including upregulating immunoglobulin production, downregulating B lymphopoiesis,[78] and altering the galactosylation[79] and sialylation of autoantibodies.[80] A direct role for sex hormones in the pathogenesis of Sjögren's syndrome was suggested by Ainola *et al*. in 2018,[81] when they found that androgens protected epithelial cells from apoptosis and induction of Sjögren's syndrome autoantigens as well as affecting Toll like receptor 7 and 9 expression. However, using sex hormones as a form of treatment, for example with androgen treatment to ameliorate autoimmunity, have for the most part not

proven successful (reviewed in: [82]), but targeting other members of androgen signaling pathways or utilizing the immunological effects of androgens in other ways warrants further study.

Outside the realm of sex-hormones and environmental factors, genetic factors can be a potent modulator of sex-differences in autoimmunity. Carriers of two X chromosomes (females and individuals with Klinefelter syndrome) tend to have more susceptibility to developing systemic autoimmunity and autoimmune diseases with more female-predominance,[83,84] while carriers of only one X chromosome (males and individuals with Turner syndrome) have a higher risk of other autoimmune disorders with more male dominance,[85] (reviewed in: [86]). Also, trisomy of the X chromosome (47, XXX) was found in excess among Sjögren's syndrome patients and systemic lupus erythematosus patients.[87] Rare X chromosome abnormalities such as triple mosaicism or triplications of one arm of the X chromosome have also been shown to be present among Sjögren's syndrome patients,[88] so a certain amount of risk appears tied to X chromosome numbers.

There are several ways in which X chromosome counts could have this effect, and one of them is genetic variations on the X chromosome. The X chromosome is home to many important immune genes, such as *Toll-like receptors*, *CD40 ligand*, *FOXP3* and *IL-2RG* to name a few. Genetic studies have revealed disease associations with X chromosome loci, including variants near *Toll-like receptor 7* and others.[89,90] On the other hand, studies tying X-linked susceptibility genes and clinical features of systemic lupus erythematosus have only found moderate effects,[91] so there is more work ahead before these effects can be fully understood.

Epigenetic regulation and ineffective X-chromosome inactivation are also potential candidate causal manifestations of X chromosome dosage. *XIST*, a gene inside the X inactivation center that is essential for the initiation and spread of X-inactivation, along with some of its regulators, has been found to be overexpressed in the affected salivary glands of patients with Sjögren's syndrome.[92] The same study also found several X chromosome genes to apparently escape X inactivation specifically in tissues from patients with Sjögren's syndrome.

As most genetic risk factors will usually disseminate from carriers to female and male offspring in equal measure, the reasons for the increased incidence in females is unlikely to stem from disproportionate counts of risk alleles (outside the sex chromosomes), but rather sex-specific downstream effects. Males are overall less susceptible to both Sjögren's syndrome and systemic lupus erythematosus, but they have also been reported to suffer from a more severe form of the disease.[93] This could partly stem from milder cases being under-diagnosed in males, but is unlikely to explain the large difference in frequency observed between the sexes. A difference in the threshold for developing these diseases has also been suggested. Then, males with Sjögren's syndrome or systemic lupus erythematosus would need to accumulate a greater number of risk factors to progress to autoimmune disease, but disease severity would be higher due to this risk factor accumulation. In fact,

looking at families of males diagnosed with systemic lupus erythematosus showed increased prevalence of more severe renal disease in affected female relatives who supposedly had similar risk exposures,[94] supporting the theory that in high risk families with an accumulation of genetic and even environmental factors, more males would pass the threshold and develop disease. This has been further evidenced in systemic lupus erythematosus studies using genetic risk score aggregation methods, where sex has an opposite effect in highly affected patients compared to other patients (seen for the 'renal' subset compared to the less affected 'SLE' subsets in: [95]), and men overall require a higher number of risk alleles to develop disease.[96]

The factors mentioned above are not mutually exclusive, and likely function in parallel to some extent. One possibility is an interplay between genetic variants and sex-specific factors like hormones. Sex hormones like estrogen can drastically influence gene expression by signaling through receptors that are expressed in a wide variety of cell types, including immune cells (reviewed in: [97]). Estrogen receptors also function as transcription factors to regulate transcription, so differences in estrogen receptor signaling could impact how genetic polymorphisms could influence disease. Be it directly through such a mechanism, or via any environmental or other sex specific influence, genetic risk variants might elicit a sex-specific response on gene-expression, generating so called sex-expression quantitative trait loci (sex-eQTLs).

In summary, there is a known sexual dimorphism in most autoimmune diseases, particularly prominent in systemic autoimmunity. This dimorphism might stem from a variety of factors, alone, or more likely in combination including: Sex hormones, environmental factors, and genetics (Figure 2). This field of study has gained much needed momentum in recent years, but the intricacies of how, when, where, and why our sex dictates the way our immune systems react to stimuli remains unclear.

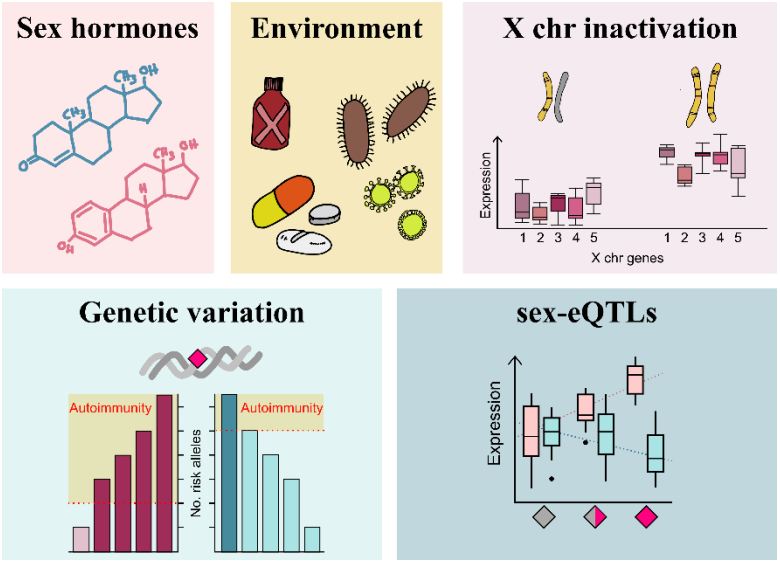

Figure 2: Summary of potential influences on sexual dimorphism in autoimmunity

1.1.4 Interferons

Interferons are a family of virus response proteins released by the host to fend off a virus infection. There are three families of interferons - type: I, II and III, each with different characteristics and binding to different receptors (Figure 3). The type I interferons include: interferon α, which in humans is encoded by 13 different *IFNA* genes, β (encoded by *IFNB*), interferon ε (*IFNE*), interferon κ (*IFNK*) and interferon ω (*IFNW*), and all binding the *IFNAR1* and *IFNAR2* encoded type I interferon receptor. The only type II interferon is interferon γ (*IFNG*). The type III interferons are the different interferon λ (*IFNL*), the last to be discovered and least known of the three.[98] The large range of different type I interferons makes it difficult to measure their levels directly, however, signaling through the type I interferon receptor leads to a signaling cascade that results in the expression of a many interferon-response genes and their expression is generally easier to assess as a proxy for type I interferon activity.[99] Under normal circumstances, type I and III interferons are necessary for protective immunity against viruses, and type II interferons for anti-mycobacterial responses (reviewed in: [100]), but dysregulation of all families of interferons has been seen in patients with autoimmune diseases.[101]

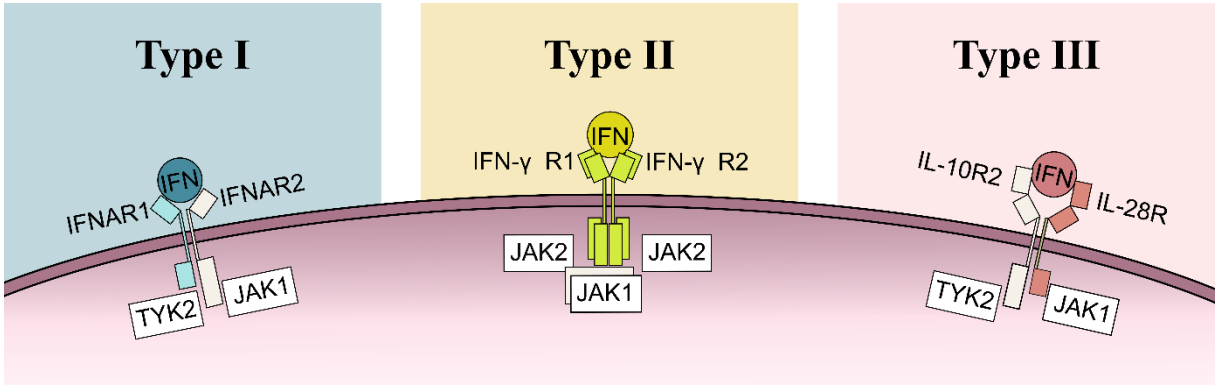

Figure 3: Schematic view of the three interferon family receptors for type I, type II and type III interferons, modified from.[102]

Interferons are viral response proteins upregulated in patients with systemic autoimmunity, but the reason for this upregulation is not fully understood. Monogenic interferonopathies hint at an explanation. Aicardi-Goutièrs syndrome is caused by mutations in the Three-Prime Repair Exonuclease *1* (*TREX1*) or ribonuclease H2 genes[103] (*RNASEH2A, RNASEH2B, RNASEH2C*) which lead to an accumulation of nucleic acids, an induction of type I interferons and inflammation (reviewed in: [104,105]). In such cases, mutations in single genes can cause an induction of interferon responses and a phenotype that in some ways resembles systemic lupus erythematosus. That shows that interferons can be induced without viruses, and cause inflammation and autoimmune-like symptoms on their own.

Patients with Sjögren's syndrome often have systemic activation of interferon production and interferon responses.[106] In patients with Sjögren's syndrome, B cells have been shown to express prominent type I and type II interferon signatures,[107] plasmacytoid dendritic cells - the main type I interferon producing cells - are activated to produce higher levels of type I interferons compared to cells from healthy controls[108] and minor salivary

gland tissue has been shown to express type III interferons[109]. Signs of activation of type I and II interferons have been found in 50-60% of patients with Sjögren's syndrome,[7,8,110] and coincide with an overall more affected phenotype. The effect of the apparent type III interferon activation in Sjögren's syndrome or systemic lupus erythematosus is still being studied.[101,111] The varied influence interferons might have in the pathogenesis of Sjögren's syndrome was recently reviewed by Bodewes *et al*.[112] and recent updates specifically in.[102]

1.2 CONGENITAL HEART BLOCK

Anti-Ro or anti-La autoantibodies are associated with Sjögren's syndrome and impart an increased risk of both lymphoma and cardiovascular disease.[113] However, these autoantibodies can also cause a passively acquired autoimmunity, the neonatal lupus syndrome, in fetuses exposed to maternal autoantibodies *in utero*. The neonatal lupus syndrome can present as a photosensitive rash, elevated liver enzymes or cytopenia in a neonate or infant, which clear once the autoantibodies are no longer present, and as a complete, permanent atrioventricular (AV) block. If the complete AV block develops, the mortality is high, with internationally reported figures of conservatively treated cohorts around 20-30%.[114-116] Most fatalities occur *in utero* or during the first year of life. Those who survive often need a pacemaker soon after birth or in their first few months. Affected neonates are also smaller at birth,[117] and more likely to develop attention deficits and other neurodevelopmental impairments later in life,[118] so further study, and particularly finding preventive treatment might improve patient quality of life, in addition to reducing the mortality rate.

Like other antibodies, anti-Ro/La autoantibodies are passively transferred across the placenta after the first trimester where their presence is associated with inflammation, fibrosis and a loss of conduction through the AV node.[119] This only happens in 1-2% of exposed individuals,[120] and the recurrence rate in subsequent pregnancies is only 12-16%.[121-123]

So far, there is no preventive treatment for congenital heart block, however, early treatment with fluorinated steroids may have promise as a treatment for those who are affected.[123,124] Hydroxychloroquine treatment is commonly prescribed to patients with Sjögren's syndrome or systemic lupus erythematosus, and its use during pregnancy has been suggested to reduce the risk of congenital heart block,[125,126] however, in order to treat and prevent congenital heart block, the central issue is identification of which individuals are at risk.

1.2.1 CHB genetics

The low recurrence rate of congenital heart block suggests that factors other than maternal autoantibodies might increase the risk of an AV block forming in an exposed fetus. While factors such as the levels of maternal autoantibodies,[127,128] season of birth, and maternal age have been suggested,[121,129] fetal risk factors emerge as likely candidates. As the autoimmune

AV block develops *in utero*, the most likely fetal risk factors would be genetically driven. The main findings of genetic associations thus far will briefly be outlined below.

The most prominent genetic associations identified, and first to be discovered, have been with variants in the *HLA* locus. In 2010, genetic associations with variants in the *HLA* region and any cardiac manifestations of neonatal lupus were published, indicating regions near *MICB* as the most significantly associated.[130] The same study found several suggestive associations outside the *HLA*, but none passed the genome-wide significant cutoff. Using family based approaches, associations with both class I and class II *HLA* alleles have also been described, including HLA-Cw*06, HLA-DQB1*06 and HLA-DRB1*13.[131-133] Due to the rarity of the disease, genetic studies on a larger scale are practically difficult, but might prove necessary to identify additional variants that confer fetal genetic susceptibility.

1.2.2 CHB and interferons

Individuals with systemic autoimmune diseases can show signs of systemic interferon activity. Sera containing anti-Ro autoantibodies can induce type I interferon production by PBMCs in cell culture,[134] and signs of a correlation between the interferon activity and a risk for developing neonatal lupus have been suggested after children born to autoantibody-negative mothers receiving alpha-interferon therapy have developed neonatal lupus.[135] Much like the fetuses exposed to autoantibodies and interferons due to maternal autoimmunity, only a fraction of fetuses exposed to interferon treatment *in utero* develop neonatal lupus, however, while the interferons alone might not induce congenital heart block in all exposed individuals, they likely contribute to disease pathogenesis in susceptible individuals.

Several studies have since supported a relationship between interferons and the pathogenesis of congenital heart block risk. Studies have shown that mothers of children affected by congenital heart block have higher levels of type I interferons than mothers of unaffected children.[136] The mothers also had increased expression of *SIGLEC1*, a suggested biomarker for interferon an autoimmune activity.[8] Both *SIGLEC1* and type I interferons have been found in samples of affected cardiac material[137] and interferons have been suggested to affect fetal fibroblasts to facilitate tissue injury.[138] Lastly, using single-cell RNA sequencing of a congenital heart block affected fetal heart, most cells were shown to upregulate interferon response-genes compared to healthy controls.[139] However, much remains to be understood about the direct role interferons play in tissue damage and disease pathogenesis.

2 RESEARCH AIMS

The aims for the projects in this thesis were to identify associations and interplay between genetic and biological factors that might influence susceptibility to, or the disease progression of, systemic autoimmune diseases, with a focus on Sjögren's syndrome and its comorbidities. This included the study of factors such as autoantibodies and sex, and their relationship with genetic susceptibility factors for systemic autoimmune disease, and the elucidation of potential functional consequences of these associations. Moreover, examination of systemic autoimmunity in the context of pregnancy and the detrimental effects of maternal autoimmunity on fetal immune activation, with a specific focus on autoimmune congenital heart block were part of the studies.

2.1.1 Specific aims

- To identify genetic associations with Sjögren's syndrome, and to identify potential subgroups of patients and means to distinguish them in terms of genetic variation and potential biomarkers (*paper I*).

- To elucidate the interplay between sex and genetic polymorphisms relevant for the expression of genes in loci associated with autoimmune disease, to identify genes that are regulated in a sex- and disease-specific manner (*paper II*).

- To elucidate the function and potential role of the novel autoimmune associated gene, FAM167A/DIORA1 in Sjögren's syndrome (*paper III*).

- To identify novel genetic associations with autoimmune congenital heart block (*paper IV*).

- To elucidate the effects of maternal autoimmunity, particularly autoantibodies, on immune activation in neonates in utero, focusing on interferons and their downstream effects (*papers V and VI*).

3 MATERIALS AND METHODS

In this chapter, some of the methodologies chosen for projects described in this thesis will be briefly discussed. Detailed listings of all the methods used for each project can be found in the attached papers and manuscripts.

3.1 PATIENTS AND CONTROLS

To identify and study different subgroups of Sjögren's syndrome patients, we collected a large cohort of patients from Sweden and Norway and generated a detailed database of clinical information and coupled it with large scale targeted sequencing. The main advantage of this was providing the unique ability to couple distinct clinical manifestations with genetic polymorphisms on a much bigger scale than what had been done before. In this study, the patient cohort was coupled with population controls from both Sweden and Norway, including both blood donors and other population controls. The downside to this arrangement is that the controls are not matched specifically for age and region of origin, however, bioinformatics methods were used to quality control the patient and control cohorts and remove any outlying or related individuals from the study (*paper I, supplementary figure S1*). The specific quality control steps used are detailed in the attached supplementary (*paper I, supplementary material, Methods*).

We collected clinical information related to the Sjögren's syndrome diagnosis for all the included patients. This included variables such as age at disease onset, age at diagnosis, presence of various autoantibodies, extraglandular manifestations and lymphoma. This information had been collected center by center during the course of the diagnostic and/or treatment process for each patient and not systematically measured or collected for the sole purpose of the study. Therefore, variables of interest such as cryoglobulins or levels separate anti-Ro52 and anti-Ro60 were too poorly covered in our dataset to be of use. This manner of data collection also means that a particular subtype of patient might be more represented with clinical information, namely the patients missing the prototypic anti-Ro/La autoantibodies. To meet classification criteria in the absence of these autoantibodies, patients would have to present with positive minor salivary gland biopsy to qualify for the Sjögren's diagnosis, and thus, salivary gland biopsies are completely covered in this group. In light of that, we excluded poorly covered clinical variables in downstream analysis. The anti-Ro/La information was present for all the patients included. For the more severe phenotypes such as lymphoma and interstitial lung disease, data collection would automatically skew towards more coverage for the affected patients, with the unaffected ones being less likely to be registered unaffected but the affected ones noted specifically. Despite this, we opted to not include variables with proportionally high levels of missing data (with a cutoff of 50%), and variables such as interstitial nephritis could not be included in the study.

3.2 GENETIC ANALYSIS

To perform a genetic association study for Sjögren's syndrome, using a targeted sequence capture array (the *'Immuno-array'* described in: [140]), we targeted 1853 genes selected based

on their known or indicated roles in immunological or autoimmune diseases. While this method is then limited to the targeted regions, this method provided much better coverage for those loci than other methods in large scale use at the time. While whole-genome sequencing would have been a better choice, it was not a feasible option at the time, however, this method allowed for a close examination of the selected regions in a large scale and high quality to examine associations between Sjögren's syndrome and known or suspected loci with immunological or autoimmune function.

The Immuno-array was designed to capture the coding exons, 5' and 3' UTRs, potential promoter regions, and splice sites, as well as nearby regions of mammalian conservation (see *paper I, supplementary materials,* for details), in regions with potential function in autoimmunity. This way, potential coding variants, as well as regulatory variants with particularly high impact near the previously associated loci could be identified for further study. While the upside of this arrangement was the added coverage, interpretation and quality control of the data has some interesting aspects. Targeted methods are, by definition, more likely than others to detect significant associations. Therefore, determining an appropriate cutoff for significance can be debated. We chose the Bonferroni corrected cutoff (with α of 0.05), $p<8.7*10^{-7}$ (based on 57,768 independent variants tested). Since this cutoff is arguably strict, we also considered suggestively associated variants ($p<1*10^{-5}$), hopefully minimizing the type II errors in the analysis. The data collected using the Immuno-Array makes analysis of rare variants and their potential association with Sjögren's syndrome possible, however, that analysis is not included in the present study, and will not be discussed here.

The prevalence of congenital heart block is one in 23.000 in Sweden[141], so even a cohort collected in a population-based manner will be of limited size. Therefore, we reached out to centers in Norway, Finland, Greece and Italy, and formed a large cohort of patients for genotyping and analysis. This allowed us to collect more patients to increase the power for a genome-wide association study, however, collecting patients from diverse geographical origins can cause issues. To circumvent this, we used principal component analysis to exclude patients who were outliers from the main patient group, and a genetic relationship matrix (GRM) to control for variation within the cohorts tested. The GRM was generated using GCTA (cnsgenomics.com/software/gcta[142]) and used as a covariate for a mixed-linear-model association analysis. This way, we were able to find loci associated with congenital heart block at a genome-wide level, with minimal inflation of the *p* values.

Families affected by congenital heart block have a moderate likelihood of containing more than one affected child. Traditional methods of case-control genetic association studies, using linear or logistic regression, therefore require a significant reduction in the already small cohort sizes, since related individuals must be removed prior such analysis. To circumvent this, mixed model approaches have been implemented in many studies where relatedness between samples can be expected, particularly in twin studies. Using a cohort of congenital heart block cases that included affected first degree relatives allowed us to boost

the size of our cohort considerably and subsequently to identify novel associations using mixed linear model association methods.

Any genetic association study attempted for congenital heart block will be complicated by maternal autoimmunity and the associated genetic variation. Mothers carrying anti-Ro/La autoantibodies often carry genetic variants such as *HLA* risk alleles that predispose them to autoimmunity and by Mendelian law, half of their offspring will inherit some of their risk alleles. Comparing the affected children to healthy controls then poses the risk of detecting maternal autoimmune risk alleles that may or may not contribute to the risk of congenital heart block. One method used to circumvent this is the transmission disequilibrium test (TDT) that has been applied previously in this setting on affected individuals and their parents.[131,133] One of the most important strengths of the TDT test is its robustness in samples from mixed populations, as it is not sensitive to differences in population structure, however, the TDT test requires heterozygosity in the parents to adequately detect transmission disequilibrium with serious implications in terms of statistical power. Therefore, while the TDT will account for maternal autoimmunity better than most other association tests, it will quickly reach its limit in terms of power to detect new associations. Thus, we selected the mixed linear model case-control association testing as a logical addition to previously published family association studies to detect new genetic associations with congenital heart block.

3.3 EQTL ANALYSIS

To look for sex influenced eQTL effects, we used the following linear model in R: lm(Expression ~ SNP + Sex + SNP*Sex), where "Expression" represents the Z score normalized expression of the gene tested and "SNP" represents the "0", "1" or "2" coded genotype. Thus, we measure the influence of genotype, sex and potential interaction between genotype and sex on the expression of the gene in question. What is missing from this equation due to the lack of availability of that information, is age. For optimal results, the model should have included the effect of age on the expression of the genes in question. Also, a limiting factor for the variants tested for sex*SNP interaction eQTL effects was including only healthy controls, since the disease associated alleles are scarce in healthy controls due to their disease associations. This had the most notable impact in the *HLA* region on chromosome 6 where insufficient allele counts were available for testing. Ideally, testing for sex-influenced eQTLs for disease associated variants should be performed in both healthy controls and patient samples, and including more control variables such as age.

3.4 ANALYSIS OF INTERFERON ACTIVITY

To examine the influence of type I interferons in our study, we have utilized several methods. One of the most common ones, as mentioned in the introduction (Interferons), is to measure downstream activity of type I interferons by assessing the expression of interferon induced genes. This is done because of the wide range of type I interferons, mainly interferon-alpha, that can be difficult to collectively measure but signal through the same receptor. For this, we

calculate the so-called interferon score, by using a range of interferon induced genes (*IFI44, IFIT1, IFIT3, IRF7, ISG15, LY6E, MX1, MX2, OAS1, OAS2, OASL, PLSCR1* and *STAT1*) and calculating the score for each individual included as follows (described in: [143]):

$$\text{IFN score}_{\text{sample}} = \sum_{i=1}^{n} \frac{\text{Gene i}_{\text{sample}} - \text{mean Gene i}_{\text{HC}}}{\text{SD(Gene i}_{\text{HC}})}$$

We have shown that this method is quite robust and can be measured in a number of different ways with a consistent result.[144] However, to examine the release of interferon alpha from neonatal cells exposed to antibody-containing plasma, we employed the DELFIA method which has been previously described.[145] Several methods of direct measurement of interferon alpha have been used in the literature, including modified ELISA methods and the SIMOA assay.[146] We have extensive experience using the DELFIA method and deemed it was appropriate for this purpose.

In order to assess if fetal cells were in fact capable of responding to maternal autoantibody containing plasma by releasing type I interferons, we cultured fetal PBMCs in the presence of autoantibody containing plasma and healthy control plasma (*paper V, figure 4*) and used DELFIA method mentioned above for interferon-alpha detection. This method allowed us to show, for the first time, that fetal cells can and will respond to plasma containing anti-Ro/La by releasing interferon. While the induction of type I interferons by anti-Ro/La autoantibodies has been shown before (see,[134] and others), we realize that this experimental setup does show a direct effect of the autoantibodies on type I interferon release, as other mediators in the plasma could hypothetically have the same effect, and to demonstrate a direct effect, the autoantibodies would have to be purified from the plasma before culture with the PBMCs. However, we could unequivocally show that the autoimmune plasma, containing autoantibodies known to induce type I interferons, could induce interferon-alpha production in fetal PBCMs, while plasma from healthy controls did not.

3.5 ETHICAL CONSIDERATIONS

When studying human disease, there are multifaceted ethical considerations that must be made. Where relevant, all the studies included in this thesis were approved by the relevant ethical committees, and ethical considerations have been taken into account for both planning and execution of each of the studies.

Any study collecting information about an individual's genetic and/or disease history must consider data security and secure information handling as a priority. Extensive steps have been taken to encrypt and protect the clinical data collected for all the cohort studies included in this thesis, and access to the data is strictly controlled. Informed consent was obtained for all participants. When studying human disease, there are numerous other considerations that need to be addressed.

Autoimmune diseases often entail progressive tissue damage leading to loss of function of affected organs, pain and morbidity for the affected individuals. This holds particularly

true for the study of congenital heart block, where the loss of function of the affected organ - the atrioventricular node - increases morbidity and mortality for the affected individuals considerably. Taking steps to better understand the causes and drivers of disease pathogenesis in order to make diagnosis or treatment more efficient could potentially reduce the level of tissue damage and pain the patient is exposed to. The potential for improved treatment, diagnostics or even a cure for autoimmune diseases provide a potential payoff for any patient that greatly outweighs the relatively minor cost of donating information and material for a genetic study such as the genetic association studies included in this thesis.

Aside from the increased risk of congenital heart block, little is known about the effects of maternal autoantibodies in anti-Ro/La exposed pregnancies (with or without congenital heart block). We invited any at-risk pregnant woman being treated in the greater Stockholm area to participate in our study. Participation was entirely optional, and participants provided their informed consent, noting that discontinuation in the study could happen any time without any effect on the clinical monitoring or care during pregnancy. Peripheral- and cord-blood from mother-neonate pairs were collected at the time of delivery according to routine procedure, with minimal risk to the mother. As the neonatal sample was collected from cord blood post-partum, the neonate is not at any risk during the sample collection. Since ethical permits for the suggested studies are approved, the consensus is that these projects are worth pursuing.

4 RESULTS AND DISCUSSION

4.1 GENETIC ASSOCIATIONS WITH SJÖGREN'S SYNDROME

4.1.1 Targeted sequencing of autoimmune loci in Sjögren's syndrome patients and controls

While updated methods of genotyping have made larger and more detailed studies of genotype-phenotype associations possible in the last decade or so, much remains unknown about the disease pathogenesis and susceptibility of many autoimmune diseases such as Sjögren's syndrome. Efforts have been made to collect large cohorts of patients to increase the power to detect disease-related polymorphisms, and all that available data has revealed a disease heterogeneity more pronounced than previously expected. To identify subgroups of Sjögren's syndrome patients to use for further study, we used a large, well characterized cohort of Scandinavian patients with Sjögren's syndrome and compared them with population controls. We analyzed both clinical and genetic data to identify subgroups based on clinical information, and examined their association with specific genetic markers and phenotypes (*Paper I*).

Using a principal component analysis of the clinical information collected, we were able to identify clear subgrouping in the patient cohort with the most distinct variable associated with the first principal component being the presence or absence of the anti-Ro and/or La autoantibodies. We tested this grouping variable on the genetic data, and found that using only the patients carrying the anti-Ro/La autoantibodies and comparing them with controls gave more significant associations with genetic variation in the *HLA* region (p $2.2*10^{-62}$) than using the whole cohort (*HLA-DQA1* p $1.4*10^{-46}$), despite a loss of power by reducing the size of the cohort. This was true for all three independent signals within the *HLA* locus found to be associated. Looking at the anti-Ro/La negative subgroup and comparing them with healthy controls, we found no genetic association with variation in the *HLA* region, and overall no genetic variants passed the significance threshold.

The association between *HLA* and anti-Ro/La positivity has been described before.[147-150] However, we were able to detect different sub-phenotypes of Sjögren's syndrome associated with the different variants within the *HLA* region, with variants near *HLA-DQA1* (rs6933289) and *HCP5/HLA-B* (rs3099839) correlating positively with younger age at onset, lymphadenopathy, major salivary gland swelling, hypergammaglobulinemia, other autoantibodies and more. These findings correlate well with previous publications, where younger age at onset was associated with more autoantibodies and an overall worse prognosis.[24] We also found that an independently associated variant near *HLA-DRA* (rs7197) was more specific for anti-Ro autoantibodies and leukopenia and not the other extra-glandular manifestations or autoantibodies. This way, we could show that the specific phenotypes dictated by autoantibody-positivity have a genetic basis that should be distinguishable not only when autoantibodies can be detected but as early as birth. The predictive value of such genetic information remains to be understood, but knowledge of the

disease-associated genotypes - potentially used together with other biomarkers - opens for possibilities for preventive or early-stage treatment.

In our study, we found that the presence of either anti-Ro or anti-La autoantibodies correlated with both clinical and genetic variation in Sjögren's syndrome, however, there are varied opinions in the field about the benefits of grouping the autoantibodies together in this manner. This group encompasses three autoantibodies in varied combinations; anti-Ro52 and anti-Ro60 (collectively referred to as anti-Ro), or anti-La. Each of these autoantibodies, alone or in combination, have been associated with separate clinical manifestations,[29,151] with the combination of all three being most common in Sjögren's syndrome compared to other diagnosis,[29] and isolated anti-La with a more limited phenotype.[30,31] Single positivity for either anti-Ro52 or anti-Ro60 can have varied clinical presentations,[152,153] however, the combination of the two, with or without anti-La, is much more common in patients with Sjögren's syndrome than other or no diagnosis. This would suggest that measuring single positivities for these autoantibodies is important for diagnostic purposes and/or other autoimmune or connective diseases. However, within the context of established Sjögren's syndrome, the presence of anti-Ro52/Ro60 with or without anti-La, compared to negativity for all three, will associate with a more severe disease phenotype.

For a patient with symptoms characteristic of Sjögren's syndrome, being correctly categorized into one of the subgroups discussed above will be important for monitoring of disease progression and appropriate assessment of the risk of severe and potentially life-threatening manifestations. Ideally, those more likely to develop severe disease could receive targeted treatment, however, following a wave of failed clinical trials,[15] good treatment options for patients with Sjögren's syndrome remain few and far between (reviewed in: [154]). Biologic treatment has proven successful for some of the most severe of extraglandular manifestations,[155] however, the main symptoms of Sjögren's syndrome - dryness of the eyes and mouth with debilitating fatigue - remain only topically treatable.[156] The failure of the clinical trials is thought to be due to patient heterogeneity as well as a lack of measurable disease outcomes,[157] although drug inefficacy for Sjögren's syndrome is certainly also an option. The association we found with anti-Ro/La autoantibodies in the presentation, progression and outcomes of Sjögren's syndrome could be of great benefit for defining patient groups eligible for clinical trials.

Outside the *HLA* locus, we also identified associations with variants near *interferon regulatory factor 5* (*IRF5*), *Mitogen-Activated Protein Kinase Kinase 2* (*MAP2K2*) and *Glutamic-oxaloacetic transaminase-1* (*GOT1*). The association with variants near *IRF5* and Sjögren's syndrome has been published previously,[43,44] however, we found that the association with *IRF5*, like the association with *HLA*, were stronger when comparing only the anti-Ro autoantibody positive patients to controls compared to the full cohort of antibody-positive and negative patients. The *GOT1* and *MAP2K2* loci however, did not improve when we removed the anti-Ro/La negative patients from the analysis, and *MAP2K2* association did not pass the significance threshold in the sub-group analysis while the *GOT1* association

merely weakened. We then attempted to replicate these findings in a smaller Scandinavian cohort, but only found associations with the *HLA* and *IRF5.* The replication was however based on much smaller material (using 177 patients and 7672 Swedish population controls) so it is possible that the lack of replication is due to a lack of power in this case, and larger cohort studies might shed light on these associations and their potential role in the disease in the future.

In summary, we used a large cohort of Sjögren's syndrome patients and population controls and collected detailed clinical and genetic information for genetic association studies. Using the clinical information collected, we found clear signs of distinct patient subgroups in our cohort, best identified by the presence or absence of anti-Ro/La autoantibodies. The identified subgroups differed in their clinical presentation, with anti-Ro/La being linked to younger age at Sjögren's syndrome onset and several extraglandular manifestations. Also, there were clear differences in the genetic associations with Sjögren's syndrome in the two groups - with the antibody carriers more often associating with both variants in the *HLA* locus and *IRF5*, but the patients not positive for these autoantibodies not showing any significant associations. These findings have potential benefits for both diagnosis, management and hopefully clinical trials for Sjögren's syndrome.

4.1.2 Sexual dimorphism in the expression of genes associated with autoimmune disease risk

Systemic autoimmune diseases such as Sjögren's syndrome and systemic lupus erythematosus show a prominent sexual dimorphism in their frequency and severity. Genetic association studies have found polymorphic genetic loci to be associated with these diseases, however, most of the disease associated polymorphisms are found in both males and females in the population in equal measure. Exploring the hypothesis that such polymorphisms may however lead to different functional effects if carried by a man or a woman, we explored potential influences of sex on the expression of genes near polymorphisms associated with either disease; in essence analyzing interactions between disease-related genotype and sex on gene expression using sex-eQTL analysis. We found that in CD19+ B cells, seven loci had genes with differential expression in males versus females depending on the presence of a nearby risk genotype (*paper II*).

In a recently published study using the large amount of data collected in the Genotype-Tissue Expression (GTEx) project, the impact of sex on eQTL effects in different tissues was explored.[158] One of their main findings was that, while widespread, sex-eQTLs are very tissue specific, with a fair percentage of the variant-gene pairs identified having an effect in only one of the 44 tissues tested. While we examined fairly homogenous sorted cells in our cohort, the GTEx sex-eQTLs are based on whole tissues. However, we were able to replicate a *genotype*sex* interaction for one of the sex-eQTLs identified in our analysis between *PXK* and rs60612015 in adipose tissue, using a proxy for rs6445975 (r^2 0.647) (Table 1). A further three variant-gene pairs from the original list of variants were discovered to have eQTLs in the GTEx data that were only significant in one sex and not the other, while

not reaching significance for the *genotype*sex* interaction term (Table 1). Both *CTSB* and *PXK* also had significant sex-eQTLs detected in several tissues for variants outside the autoimmune associated loci (not shown), suggesting widespread influence of sex on the expression of those genes.

Table 1: Replication of sex-eQTLs in GTEx

			*Genotype*sex*		*Females*		*Males*	
Variant id (Original variant, R^2)	**Gene**	**Tissue**	***P***	**Slope**	***P***	**Slope**	***P***	**Slope**
rs2814955 (rs11755393, *0.926*)	*UHRF1BP1*	Artery (Tibial)	**3.9E-03**	-0.127	**3.8E-32**	0.634	**5.6E-31**	0.774
rs9296128 (rs11755393, *1.0*)	*UHRF1BP1*	Thyroid	**0.038**	0.128	**6.9E-13**	0.431	**3.6E-07**	0.334
rs60612015 (rs6445975, *0.647*)	*PXK*	Adipose (Visceral)	**0.040**	-0.146	**1.3E-09**	-0.470	**2.3E-05**	-0.36
rs558245864	*FAM167A*	Spleen	**0.047**	0.266	**4.8E-12**	1.64	**6.2E-13**	1.24
rs4728142	*IRF5*	Heart (Atrial Appendage)	0.083	0.111	**9.2E-05**	0.334	0.066	0.137
rs6445975	*PDHB*	Pancreas	0.11	-0.131	0.62	0.0424	**2.7E-03**	0.249
rs429503 rs2732552, *0.956*)	*CD44*	Nerve (Tibial)	0.36	0.0425	**4.8E-04**	0.179	0.054	0.0822

The variant-gene pair detected in both our analysis and in the GTEx data, *PXK* and rs6445975 (or proxy rs9296128) showed downregulation of *PXK* expression in both sexes in GTEx. We also found that the genotype*sex had a negative β value in our analysis, however, in B cells, the expression of *PXK* was upregulated in female carriers of the risk allele while male expression levels were largely unaffected (*Paper II, figure 2C*). We included variants in the *PXK* region because of their association with systemic lupus erythematosus, but the 3p14.3 locus has also been associated with a number of other diseases and phenotypes, including systemic scleroderma, rheumatoid arthritis, acute myeloid leukemia and various measurements such as platelet count, hematocrit, hemoglobin and serum IgG (GWAS catalog[159]). *PXK (PX Domain containing Serine/Threonine Kinase like*) is a gene found in most tissues, with an elusive function and no obvious disease causing features at first glance. The locus has been extensively studied in the context of its association with systemic lupus erythematosus, and the neighboring gene *ABHD6* was found to be most profoundly affected by the disease associated variants.[160] In addition to the sex-eQTL effects we detected in *PXK*, we also found cis-eQTL effects of the disease associated variants on yet another gene in the locus, *DNASE1L3*. However, the associated signal has been mapped to one exon of *PXK*, with a functional implication in B cell receptor internalization being affected in disease allele carriers,[161] so variation in this locus appears to have a broad effect on gene expression in a sex-dependent and independent manner.

The *FAM167A/BLK* locus has been documented to have some of the most profound eQTL effects in the human genome with the most prominent effect of autoimmune-associated polymorphisms on *FAM167A* expression in B cells.[62] This was clear in our cis-eQTL analysis where we detected eQTL effects on three genes in the region, *FAM167A*, *BLK* and *FDFT1*. In the GTEx sex-eQTL data, the slope of the male and female eQTLs in spleen tissues were 1.24 and 1.64, respectively, showing the magnitude of the effect on *FAM167A* expression. However, in our sorted B cell data, there was no detectable sex-eQTL effect on *FAM167A* expression, but only on the neighboring *CTSB.* The GTEx data had detectable *FAM167A* sex-eQTLs for both the disease associated variants and variants not currently known to associate with autoimmunity (rs2572418 in thyroid tissues), however, it's possible that the difference seen there stems from a difference in cell types present in the tissues and not a direct sex-eQTL,[158] although that would not subtract from any potential biological effects. In the chromosome 8 locus, we found sex-eQTL effects on *CTSB* (*Cathepsin B*) expression with autoimmune associated variants (rs922483 and rs13277113). *CTSB* has gained interest recently due to its function as part of the entry machinery for the SARS-CoV-2 virus responsible for the COVID-19 epidemic, and a recent study concluded that *CTSB* would be an unlikely driver of sex differences in disease progression seen in the COVID-19 pandemic.[162] However, our findings would contradict that conclusion and the increased expression of this SARS-CoV-2 entry machinery protein only on male B cells could be of interest in that context. In the GTEx data, there was also an exonic *CTSB* variant (rs4839) that only upregulated *CTSB* expression in lung tissues from males and not females carrying the variant, this locus has not been associated with systemic autoimmune disease but indicates a wider influence of sex on *CTSB* expression and potential implications in COVID-19 responses.

Among the other loci we identified sex-eQTLs for, the most prominent effects were on *SLC39A8* expression in the disease associated *BANK1* locus in chr4q24, and *CD74* in the *TNIP1* locus in chr5q33.1. The disease associated variants tested had opposite effects on gene expression in females compared to males, with the least disease associated genotypes causing lower expression in females but higher in males compared to carriers of the non-risk alleles. *SLC39A8* (*Solute Carrier Family 39 Member 8*) is a metal ion transporter with a plethora of functions via control of ion transport in various cells. The function of *SLC39A8* in the immune system is bound to the secondary effects of the ions it transports, making it an important regulator of immune function primarily through zinc metabolism.[163] Knocking out *SLC39A8* leads to early embryonic lethality, and reduced expression of *SLC39A8* is linked to a wide variety of traits (reviewed in: [164]), so the implications for the reduced expression in females carrying the autoimmune risk allele could be widespread. *CD74* is a chaperone important for MCH class II antigen processing and it has been implicated in the pathogenicity of several autoimmune diseases (reviewed in: [165]). Reducing *CD74* levels in mouse models of systemic lupus erythematosus ameliorates the inflammatory symptoms, particularly in the kidneys.[166] Female B cells showed reduced levels of *CD74* in risk allele carriers, but increased in males. In systemic lupus erythematosus, males are less likely to develop the

disease, but if they do, they are more likely to get nephritis and end stage renal disease than females,[167] so a connection between the increased levels of *CD74* in males and its connection to renal function in systemic autoimmunity might be worthy of more study.

Archain 1 (*ARCN1*) in the 11q23.3 locus neighboring *CXCR5,* was upregulated in female and downregulated in male B cells carrying the autoimmune risk genotype. Loss of function mutations in this gene, which encodes a coatomer subunit of the COPI complex that coats vesicles during protein transport, cause a craniofacial syndrome and developmental delay (*ARCN1*-related syndrome).[168] *DHX9* (*DExH-Box-Helicase 9*) in the *NCF2* locus in 1q25.3 was upregulated in females but downregulated in male B cells. It has a range of important functions as a nucleic acid helicase, and its role in autoimmune processes could potentially be numerous. *DHX9* has been shown to bind specifically to inverted-repeat Alu elements and inhibits the amount of circular-RNAs in cells[169] to limit transposon activity in the genome, but transcription of Alu elements and their binding to Ro60 - the target of anti-Ro60 autoantibodies - is part of a feedback loop of type I interferon responses and initiation, a key process in systemic autoimmunity.[170] While neither of these sex-eQTLs replicated in the GTEx tissue data, both could have implications in sex-specific autoimmune disease by affecting specific processes not measurable in whole tissue samples. These, as well as all the sex-eQTLs discovered, are worthy of further study particularly in a disease setting.

In summary, we set out to discover sex-eQTL effects for *variant-gene* pairs in a set of 21 genetic loci independently associated with systemic autoimmune disease. In our published study, we discovered 7 interactions in 6 independent loci, and found 5 more *variant-gene* pairs, 3 of them in loci without sex-eQTLs in the first study, in the public GTEx sex-influenced eQTL analysis data. This would indicate that half of the loci associated with female predominant systemic autoimmune diseases have potential sex-eQTL effects, with important implications for the pathogenicity of these variants and the susceptibility and disease progression of these diseases.

4.1.3 The novel disordered in autoimmunity (DIORA) gene family

Before the publication of the first genome-wide association study for Sjögren's syndrome, the association of genetic variants in the *FAM167A-BLK* locus on 8p23.1 with disease risk had already been published.[45] This association was later confirmed in a genome-wide study[43] and numerous other studies on both Sjögren's syndrome and other autoimmune diseases (Figure 4).

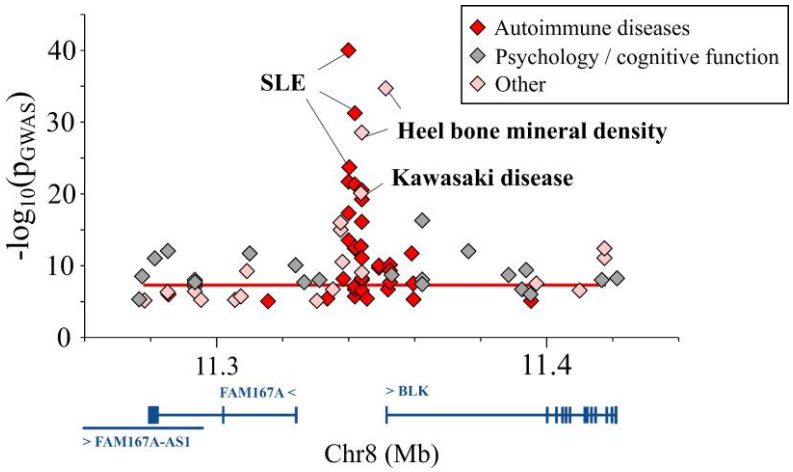

Figure 4: GWAS catalog entries for the chr8p23.1 locus
(accessed via the UCSC Table browser.[171]

The associated variants are spread over the intergenic region between *FAM167A* and *BLK* (*Paper III, Figure 1*). *FAM167A* stands for *Family with sequence similarity 167, member A*, while *BLK* stands for *B lymphoid kinase* and both are situated in an approximately 4.5 Mb long region on chromosome 8 that has been inverted in a large portion of the human population, constituting the largest polymorphic inversion in our genome, the 8p23 inversion polymorphism.[172] The autoimmune associations are more often found on the non-inverted genotype, suggesting that the inversion itself is protective.[173]

The associated signal stretches closer to *BLK*, and the known role for *BLK* in B cell function would suggest any causal effect of this genetic association to be due to effects on *BLK*, but at a closer look at the eQTL effects of these variants on nearby genes diverted attention to *FAM167A*. We analyzed human B cells for eQTL effects of the associated variants (rs13277113), and found *FAM167A* expression to be profoundly different between genotypes, with the levels in risk allele carriers being almost double that in non-risk carriers, while the eQTL effect on *BLK* expression was significant but modest towards a reduction in risk allele carriers. A study published later found that the eQTL effects for these variants on *FAM167A* were among the most prominent in the human genome,[62] with a P value of $2.48*10^{-80}$ for the eQTL effect of rs4840658 on *FAM167A* expression in B cells. They also found significant eQTL effects on *BLK* in both B and T cells.

We found *FAM167A* to be highly expressed in mouse lung tissues, followed by spleen and muscle, and specifically in B cells. More recently, fine mapping of eQTL effects on a larger scale have become available through GTEx,[174] where one can see that although the gene is highly expressed in lung, surprisingly, the expression there does not appear to be affected by the polymorphisms to the same extent as in the immune cells (Figure 5). In the GTEx data the eQTL significance peak is visible for the whole region between the two genes in both whole blood and spleen.

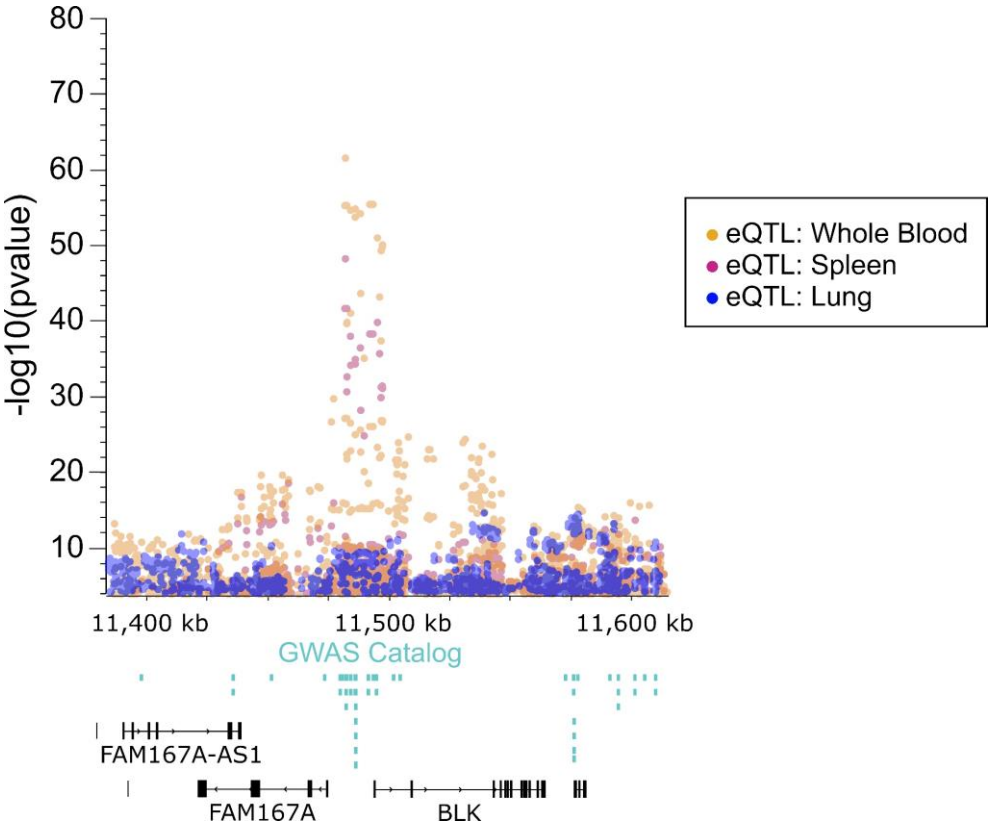

Figure 5: GTEx IGV Browser overlay for the associated signals on chromosome 8 (www.gtexportal.org *accessed on 2020-10-29*).

We later found *FAM167A* to be expressed in both B cells and plasma cells in salivary gland infiltrates from patients with Sjögren's syndrome, where it correlated positively with disease markers such as serum IgG levels, anti-Ro/La autoantibodies and salivary gland focus score,[175] further solidifying its implication in disease pathogenesis.

Aside from the prominent eQTL effect and proximity to disease associated alleles, the function of *FAM167A* was unknown. The sequence had only one gene with any sequence homology, so the pair were grouped into the *family with sequence similarity 167*, members *A* and *B*. Both genes lacked known motifs or domains, outside the "DUF3259" that grouped them together, but they were both highly preserved, with homologues even in invertebrates. The structure of both proteins is unknown, but they both have signs of a high degree of predicted disorder. Intrinsically disordered proteins have widespread roles in biological processes but do not fold into stable protein structures and instead flow and fold in a context dependent manner. This gives a level of flexibility impossible for rigid three dimensional structures seen in non-disordered proteins (reviewed in: [176]), and the importance of intrinsically disordered proteins in immune function is constantly becoming more evident.[177]

Given the importance of *FAM167A* in autoimmune susceptibility, and its apparent structural disorder, we named the gene family *disordered autoimmunity* (*DIORA)* and the two family members *1* and *2*. While our data suggests an important role for *DIORA1* in both the susceptibility and potentially even the pathogenesis of many autoimmune diseases, this does not disprove any association with *BLK*. Knocking out *Blk* in mice has only a mild B cell phenotype,[178] but Src family proteins such as *BLK* are important regulators of B cell function

in both health and autoimmunity (reviewed in: [179]), and more studies will be needed before we can fully understand the interplay between genetics and protein function in systemic autoimmune pathogenesis.

4.2 AUTOIMMUNE CONGENITAL HEART BLOCK

Autoimmune congenital heart block is an acquired, permanent heart condition that occurs during gestational week 18-24 of pregnancy in a small percentage of fetuses exposed to maternal anti-Ro/La autoantibodies *in utero*. Diagnosis is usually made *in utero*, or more rarely after birth. The incidence of congenital heart block is 1-4% in autoantibody exposed pregnancies, but increases to 12-16% if the mother has previously had an affected child. The higher recurrence rate compared to the incidence numbers when considering all anti-Ro/La positive pregnancies suggests some qualitative difference in the autoantibodies or maternal factors aside from the persistent maternal autoantibodies might increase the risk of congenital heart block. However, the recurrence is still low, so fetal susceptibility factors are also likely to be of importance. Fetal genetic factors are a logical source of risk. However, the maternal autoimmunity per se is associated with genetic profiles deviating from the normal population, which complicates the genetic analysis. Autoimmune risk genes will often be inherited from mother to offspring according to normal Mendelian laws and may thus cause higher frequencies of such alleles compared to healthy controls - and discerning true genetic associations with congenital heart block from inherited genetic autoimmune risk factors becomes difficult,[180] regardless of any role these autoimmune associated genetic variants might play in congenital heart block pathogenesis.

4.2.1 Genetic associations with autoimmune congenital heart block

Autoimmune congenital heart block is a rare disease, affecting 1:23,000 births in Sweden.[141] The rarity makes it difficult to collect enough cases for genetic analysis, so in addition to collecting as many cases as possible in Sweden we expanded our search and collaborated to collect congenital heart block cases from five European countries; Sweden, Norway, Finland, Greece and Italy, and compared them with healthy controls in a genome-wide association study.

Using a mixed linear model association test with a genetic relationship matrix to account for relatability, we compared 213 congenital heart block cases with 5671 population controls, analyzing 559,075 genetic variants. Two independent loci passed the Bonferroni-corrected significance cutoff of $p<8.9*10^{-8}$ (*paper IV, figure 1*), one in the *HLA* locus on chromosome 6, at $p=8.08*10^{-9}$, and another on chromosome 1, near *KCNT2*, at $p=7.4*10^{-8}$. Previous studies have described associations with variants in the *HLA* locus and cardiac neonatal lupus (including any cardiac phenotype for neonatal lupus, not only complete heart block cases[130]) and complete congenital heart block,[131-133] however, we found that the signal we discovered as associated with CHB (with the best association represented by rs6906021) was independent from the previously described signal (rs3099844, R^2 0.12). To understand if we might have a second independent signal in the *HLA* region, we performed a conditional

analysis, conditioning on rs6906021. The top variant in the conditional analysis was rs1150755, and that signal was in higher linkage disequilibrium with the previously reported variant (R^2 0.57), but the association with that signal did not reach significance after conditioning on the top *HLA* variant ($p_{cond}=4.1*10^{-5}$) but was suggestively associated in the original association test ($p= 5.1*10^{-7}$). Given the previous association, one might speculate that with a larger cohort, both signals might pass the significance cutoff, however, we could not replicate this signal in the current study.

Few genome-wide association studies with congenital heart block have been published. However, the association between neonatal lupus and/or congenital heart block susceptibility and *HLA* types has been known for a long time.[131,133,181] Therefore, we wanted to understand if the signals we discovered in the *HLA* region were tagging particular *HLA* types, and used a dataset of genotyped and *HLA* typed Swedish controls to calculate associations between our alleles and *HLA* types (*paper IV, figure 2C*). We could not find our top signal (rs6906021) to tag a particular *HLA* type in our analysis. However, the previously described associated polymorphism[130] (rs3099844), and to a lesser extent our second, less associated, signal (rs1150755) correlated well with HLA-B*08:01 and to some extent with HLA-DRB1*03. These two alleles are part of a haplotype associated with SLE and Sjögren's syndrome, and the signal may thus rather represent the maternal autoimmunity genetics. Whether our top *HLA* SNP represents a true CHB-associated signal is difficult to prove; while it does not tag any SLE or SS-related *HLA* it is noteworthy that no signals with such low p-values have been observed in CHB family investigations of the *HLA* region using transmission disequilibrium test (TDT) based analysis.[131-133]

The second significant signal we discovered was near *KCNT2* on chromosome 1. *Potassium Sodium-Activated Channel Subfamily T member 2*, or *KCNT* has also been named *SLICK* and *SLO2.1*, and encodes a potassium channel activated by intracellular sodium and chloride levels expressed in a number of cell types, including cardiomyocytes where it functions in mitochondria.[182] The locus, chr 1q31.1 has a number of interesting features in the context of congenital heart block risk. First, previous genome-wide association studies have identified suggestively associated variants in the region,[130] although, we could not find high linkage disequilibrium between our best association (rs12567147) and the previously described association (rs1890645). Second, the variants are located in an intergenic region downstream of *KCNT2*, but further downstream of these variants lies Ro60, the gene encoding the Ro60 protein to which the anti-Ro autoantibodies binds. We could however not identify any functional connection between the variants and expression of Ro60, but the proximity is interesting.

The third point of interest for the chr1q31.1 locus is the proximity to the complement factor-H genes further upstream of *KCNT2*. Using the GTEx database,[174] we identified associated variants that had eQTL effects on one of the factor-H genes, *CFHR3* (*paper IV, supplementary figure 2A*), but the *HLA* variants associated with congenital heart block also had eQTL effects on C4A expression in the heart, suggesting a broad complement

dysregulation in individuals carrying the congenital heart block risk alleles. Variants in the factor-H genetic region, independent of the associated signal we discovered, have been associated with systemic lupus erythematosus susceptibility,[183] younger age at lupus nephritis onset,[184] and serum complement (C3 and C4) levels.[185] The systemic lupus erythematosus susceptibility variant described by Zhao *et al.* (rs6677604[183]), has a similar eQTL effect on factor-H genes, including *CFHR3*,[174] so overlapping pathways could hypothetically be at play in both diseases. Overall, there are multiple potential causes for congenital heart block susceptibility in the 1p31.1 locus, and functional effects of variation in this locus warrants further study.

Systemic interferon activation is a hallmark of systemic autoimmune diseases such as Sjögren's syndrome and systemic lupus erythematosus, and interferon activity has also been implicated in congenital heart block. This will be discussed in detail in the following chapter on *papers V* and *VI.* Given the suggested role for interferon activation in the pathogenesis of congenital heart block, and the fact that fetal exposure to maternal anti-Ro/La autoantibodies is concomitant with increased interferon activity in affected neonates (discussed in *papers V* and *VI*), we examined genes near (+/-1 Mb) the associated variants in the *HLA* and *KCNT2* loci, as well as suggestively associated genes ($p<1*10^{-5}$) for signs of interferon regulation in fetal cardiomyocytes. We treated cultured primary human fetal cardiomyocytes obtained from elective termination of normal pregnancies with either interferon alpha, interferon beta or control for six hours before measuring gene expression via microarrays. We found three dominating interferon responsive genes in the *HLA* locus, *TAP1*, *TAP2*, and *TAPBP*, as well as several others including C2 and complement factor-B (*paper IV, figure 3B).* In the *KCNT2* locus, all three nearby genes were IFN-regulated - *KCNT2* was significantly upregulated after interferon-alpha treatment, *miR4735* and *CFH* were both significantly upregulated only after interferon beta treatment. We then wanted to replicate this finding in whole human tissue, so we downloaded microarray data for atrial cardiac tissue, with or without atrial fibrillation (GDS accession number: GDS1559) and calculated interferon scores using two available genes (*UBE2E2* and *STAT1*), with tissue from healthy hearts as a control, and compared the interferon score to *KCNT2* and *CFH* expression levels. The array used did not have available probes for *miR4735*. Both analyzed genes were found to correlate with the interferon score also here, corroborating our observation that interferon influences genes near variants associated with congenital heart block. The same was true for genes near the suggestively associated loci, and there was a significant enrichment of interferon responsive genes among all the genes near loci associated below the suggestive *p* value ($p<1*10^{-5}$) cutoff (*paper IV, figure 3G*).

The associated variants in chromosome 1 are downstream of *KCNT2*, and thus deciphering the influence of the variants on upstream gene expression was not straightforward. To further elucidate this, we accessed publically available promoter capture Hi-C data via CHiCP[186] and found signs of interactions between the associated region and the transcription start sites of *KCNT2* and *CFH* particularly in macrophages suggesting some influence in immune cells. We looked for differential expression of genes near associated or

suggestively associated loci between peripheral blood mononuclear cells collected from the umbilical cords of neonates born to healthy mothers, and neonates born to mothers carrying anti-Ro/La autoantibodies (without congenital heart block), and found several differentially expressed genes in both the *HLA* signal and suggestively associated regions, although neither *CFH* nor *KCNT2* were affected in these cells.

In summary, we discovered two loci significantly associated with congenital heart block susceptibility, in the *HLA* and *KCNT2* regions on chromosomes 6 and 1, respectively. Variants in both of these loci influence the expression of complement genes, and the complement factor-H encoding gene is in the associated locus on chromosome 1. Interferon responsive genes were enriched among the genes near both of the significantly associated loci, and many suggestively associated ones, indicating a potential role for the interplay between interferon activity and fetal genetic factors in congenital heart block pathogenesis.

4.2.2 Interferon activation in newborns exposed to anti-Ro/La autoantibodies

Patients with systemic autoimmune diseases, including Sjögren's syndrome, often have measurable interferon in their circulation. Systemic activation of interferon-induced genes is thus a hallmark of these diseases, often used as a proxy for type I interferon activity due to issues with direct measurements of type I interferons as previously discussed. Type II interferon, represented by the single protein interferon-γ, can be more readily measured directly. The presence of type I and type II interferons have been reported to precede the diagnosis of systemic lupus erythematosus, with type II interferons appearing before autoantibody positivity, and upregulation of type I interferon responsive genes coinciding with the induction of clinically detectable disease in that report.[187] Upregulation of type I interferon responses before autoantibody appearance and disease onset has however been found in the context of e.g. type 1 diabetes, and it is not unlikely it may occur also in systemic autoimmunity. Interferons are known to be induced by a number of pathways with potential relevance for autoimmune pathogenesis (reviewed in: [188]), including induction by anti-ribonucleoprotein autoantibodies such as anti-DNA/histone and anti-Ro/La antibodies, although the concomitant presence of priming type I interferon appears necessary.[189]

The risk of developing congenital heart block is closely linked to the presence of maternal anti-Ro/La autoantibodies. The autoantibodies of IgG class are, as other IgGs, transported across the placenta to the fetus where they have been suggested to contribute to development of the complete atrioventricular (AV) block, although the mechanism remains to be defined. Cross-reactivity with cell-surface expressed targets have been reported, but no unanimous target has been described or reconfirmed (reviewed in: [190]). At the time when we initiated this study, no one had considered that the autoantibodies may actually also contribute to spark interferon production in the baby, or that the baby expected by a mother with high interferon activation may also have high expression of interferon-regulated genes.

4.2.2.1 Interferon production in Ro/La-exposed neonates

We hypothesized that the same mechanism, involving anti-Ro/La autoantibodies, may be inducing interferon in both the mothers and the baby *in utero*. To test this, and understand if neonatal cells can produce interferon upon autoantibody-exposure, we set up experiments where cells from both control and antibody exposed neonates were cultured in the presence of plasma from neonates of anti-Ro/La positive mothers or healthy control neonates. Both groups released high amounts of interferon-alpha when exposed to the autoantibody containing plasma, but neither the cells from autoantibody-exposed neonates, nor the healthy control cells responded to the control serum (*paper V, figure 4*). Cells and plasma from autoantibody positive mothers were used as a positive control as this setup is known to yield interferon production.[189] The fact that neonatal cells can produce interferons in response to autoantibodies does not mean they do so *in vivo*, and to investigate fetal production of interferon we measured interferon levels in blood and performed flow cytometry with intracellular staining of interferon-γ. Indeed, both type I and type II interferon were detectable in plasma of neonates exposed to Ro/La autoantibodies in utero, and both their T cells and NK cells were found to express interferon-γ, even without *ex vivo* re-stimulation (*paper VI, figure 5*).

These data demonstrate that neonatal cells can produce interferon in response to autoantibody-containing fetal plasma, and that interferon is indeed produced in the fetus when exposed to Ro/La autoantibodies, although this was only evaluated for interferon-γ. In PBMCs, interferon-α is predominantly produced by plasmacytoid dendritic cells. This cell population is present in low frequencies, and establishing flow cytometry, including evaluating antibodies for flow cytometric application for interferon-α, is a current project in the research group. Interferon in the neonate could potentially also originate from the mother, directly passing through the placenta. Our observations do not exclude this possibility, and while it appears likely at least some of the maternal interferon can reach the fetus, direct evidence of mid-gestational transplacental interferon transfer is still lacking. Notably though, these two sources of interferon are not mutually exclusive, and even low levels of transfer of interferon from the mother may provide the fetal cells with the priming necessary at least *in vitro* for autoantibody-mediated interferon production by immune cells.[189]

4.2.2.2 Downstream effects of fetal interferon

To understand downstream effects of the interferon activation, and if interferon-regulated genes are affected in neonates exposed to anti-Ro/La autoantibodies *in utero*, we collected samples of blood from anti-Ro/La positive women and their neonates at the time of birth, as well as healthy control pairs, and analyzed the samples for gene expression and cellular populations (*Papers V* and *VI*). The clinical features of the individuals included in each study are listed in *paper V, table 1* and *paper VI, table 1*.

As expected, the anti-Ro/La autoantibody positive mothers had notable differences in gene expression of PBMCs, with 1795 genes being differentially regulated, both up and

down, between the two groups (*paper V, figure 1*). This effect was evident regardless of the maternal diagnosis, and rather related to the presence of the autoantibodies. As previously described,[191] the mothers also had relative T cell lymphopenia and differences in the frequencies of B cell subsets, with lower frequencies of naïve B cells and higher frequencies of memory and marginal-zone B cells (*paper VI, figure 1*).

The neonates did not differ in B and T cell frequencies between groups, however, the neonates exposed to maternal autoantibodies had higher frequencies of $CD56^{dim}CD16^{high}$ NK cells (*paper VI, figure 2*). Despite only select populations differing in frequencies, there was an even greater gene dysregulation between the neonates born to healthy control mothers compared to the neonates exposed to maternal autoantibodies *in utero*, with a majority of the differentially expressed genes being upregulated in the autoantibody exposed group (*paper V, figure 1*). Some of the differentially regulated genes were shared between the maternal and neonatal analysis, but both comparisons also had unique genes that differed between groups, so the difference observed in the neonates was not a mere mirror image of the maternal effects. Gene ontology (GO) analysis also showed different pathways being enriched in the maternal versus neonatal analysis. The differentially expressed genes in the neonates included genes belonging to interferon, immune response pathways and others, but less specific pathways such as vesicle coating and platelet derived growth factor signaling were enriched in the mother. The pathways shared between mothers and neonates on the other hand, were all virus response and interferon pathways, with the viral pathways presumably identified because of the overlap with interferon signaling (*paper V, figure 1B*). PBMCs from the neonates exposed to maternal autoantibodies also displayed upregulation of NK cell related genes (*paper VI, figure 3*). None of the neonates included were diagnosed with congenital heart block, and yet, their cells were upregulating interferon response genes to the same extent as cells from their mothers.

To further understand the extent of this interferon activation in both groups, we specifically examined GO terms related to the regulation of type I interferon production, and the responses to type I interferon. There we observed signs of interferon response genes being upregulated in autoantibody exposed mothers and neonates, suggesting that PBMCs from both groups had been exposed to type I interferons. Interestingly, cells from both mothers and neonates were also upregulating genes involved in the regulation of type I interferon production, alluding to the local production of interferons in the neonates. We next calculated an interferon score for all the samples and found significant increases in the interferon score of both mothers and neonates exposed to autoantibodies. In this context we also looked at mother-neonate pairs that had previously been excluded due to maternal immunomodulatory treatment. Contrary to what others have described,[192] we could see no significant difference in the interferon scores of mothers receiving hydroxychloroquine compared to the untreated mothers. Although the group was small, all but one mother from the treated group had interferon scores comparable to the untreated mothers. It is however possible that their interferon score before treatment was higher. The neonates of treated mothers on the other hand had significantly lower interferon scores. When comparing the interferon scores of

mothers and neonates, we could see a clear correlation between maternal and neonatal interferon scores in untreated pairs (r^2 0.87). The treated pairs did not have this correlation, suggesting that the hypothesis that interferons would cross the placenta specifically in autoimmune affected pregnancies,[193] but not healthy pregnancies,[194] might not explain the interferon found in the neonates, or perhaps that the immunomodulatory treatment prevents that.

Notably, neonates of mothers with hydroxychloroquine treatment also had reduced frequencies of NK cells compared to neonates born to untreated mothers, suggesting a protective effect of maternal immunomodulatory treatment on interferon activation in the neonates. Protective effects of immunomodulatory treatment in CHB have been suggested based on clinical experience,[125,195] and a first open label trial,[126] although this remains to be fully proven.

In addition to the type I interferon activation, type II interferon and fetal NK cell activation could provide the link to more targeted tissue destruction in congenital heart block pathogenesis. Fetal NK cells have been suggested as robust inducers of tissue damage based on findings from neonatal autoimmune ovarian disease models (reviewed in [196]). We found that neonates exposed to maternal anti-Ro/La autoantibodies had increased frequencies of NK cells, and that those NK cells had elevated intracellular interferon-gamma (*paper VI, figure 5*). As previously mentioned, genetic association studies have pinpointed genetic variants in *HLA-C* as associated with congenital heart block.[131,133] Interestingly, the identified allele (Cw*06), has a protective effect. This allele distinguishes itself by being lowly expressed, and has a more restricted peptide binding than other *HLA-Cs*, suggesting that higher *HLA-C* expression and perhaps peptide presentation capacity increases the risk of CHB. The levels of *HLA-C* have been shown to influence NK cell subset frequencies,[197] and we found that type I IFN stimulation of fetal cardiomyocytes upregulated *HLA-C* expression (*paper VI, figure 4*), suggesting a potential mechanism for the interplay of fetal genetic susceptibility, interferon activation and NK cells in congenital heart block pathogenesis.

In our studies, we only had access to biologic material in terms of cord blood from term neonates. Interestingly however, fetal cardiac tissues from terminated pregnancies in which the fetus developed CHB display interferon activity,[139] demonstrating that the interferon activation also takes place in the affected organ. This interferon has been suggested to cause injury in the affected hearts by damaging fetal fibroblasts,[138] in a macrophage dependent manner,[137] where upregulation of the activation marker *SIGLEC1* in affected fetal hearts and on maternal cells,[136] was suggested as a marker of interferon activation and high risk. We found upregulation of *SIGLEC1* on monocytes from anti-Ro/La positive mothers as well as their neonates (*paper V, figure 3*). While our findings show that anti-Ro/La autoantibody containing plasma can induce type I interferons from fetal cells, with potential implications for interferon activation in exposed fetal tissues, it also suggests that the interferons - just like the anti-Ro/La autoantibodies - are necessary but might not be sufficient to induce congenital heart block without the presence of additional pathological factors.

In summary, we found that neonates exposed to maternal anti-Ro/La autoantibodies have higher frequencies of NK cells, increased levels of interferon-alpha, intracellular interferon-gamma, and differential expression of IFN-regulated genes at birth, compared to neonates born to healthy control mothers. We also found that this increase in interferon-activity is reduced when the mothers receive immunomodulatory treatment. We also showed for the first time that neonatal PBMCs respond to anti-Ro/La containing plasma by releasing type I interferons. Our findings add to the understanding of to what extent interferon activation happens in autoantibody exposed neonates, what effects immunomodulatory treatment might have on that interferon activation, and lastly, potential fetal sources of interferon production in an autoimmune setting and how that might influence the risk of congenital heart block susceptibility.

5 SUMMARY AND FUTURE PERSPECTIVES

The work included in this thesis was focused on genetic variation associated with increased risk of systemic autoimmune diseases, primarily Sjögren's syndrome, as well as one of its more severe comorbidities, congenital heart block.

We examined the influence of genetic variants on particular disease sub-phenotypes, identifying distinct groups of Sjögren's syndrome patients and the genetic and clinical variables that identify them. We found that Sjögren's syndrome patients who do not carry the typical anti-Ro or anti-La autoantibodies have a disease more restricted to the affected glands, with fewer extraglandular manifestations, and none of the typical genetic variants associated with systemic autoimmune disease. Patients with Ro/La autoantibodies on the other hand, have significant associations with variants in the *HLA* region that vary between different clinical manifestations. Our conclusion is that stratifying patients by these autoantibodies will be important for prognostic estimations and in follow-up, as well as in clinical trials and for choosing treatment. While patients belonging to the autoantibody-positive group are more likely to suffer severe disease manifestations, further study of the autoantibody-negative group might reveal pathogenic mechanisms specific for the glandular inflammation and tissue destruction observed in these patients.

To elucidate why there is such a prominent sexual dimorphism in systemic autoimmune diseases such as Sjögren's syndrome, we identified disease associated genetic variants with a sex-specific impact on gene expression in cells important for disease pathogenesis. Further understanding the difference between males and females in this context will clarify important pathways of disease pathogenesis and potentially reveal new ways to treat or manage systemic autoimmunity.

Furthermore, we specifically examined an unknown gene affected by genetic variants associated with autoimmune susceptibility, *FAM167A/DIORA-1* and its gene family, and found important implications for this gene in disease biology. The DIORA family of disordered proteins should be further characterized, and the mechanism behind the profound effect disease associated variants have on *DIORA-1* expression warrants further study.

Additionally, we identified novel genetic associations with autoimmune congenital heart block in a case-control association study. These variants should be studied further in the context of the interplay between maternal autoimmunity and fetal disease susceptibility. Genes near the associated variants showed clear signs of interferon influence in their regulation, suggesting an interplay between interferon activation and genetics in congenital heart block susceptibility. We further characterized the interferon activation in autoantibody-exposed pregnancies and found distinct signs of interferon activity in exposed neonates as well as an overall immune activation. Our findings suggest a hypothesis where autoantibodies and interferons may initiate pathways of tissue destruction that are dampened in most individuals but escalate in carriers of genetic susceptibility factors into irreversible tissue damage and loss of conduction through the atrioventricular node. Further studies will be needed to support this hypothesis and hopefully pinpoint particular risk factors that can be utilized for diagnosis and/or treatment to prevent congenital heart block.

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7 REFERENCES

1 Björk, A., Mofors, J. & Wahren-Herlenius, M. Environmental factors in the pathogenesis of primary Sjögren's syndrome. *Journal of internal medicine* **287**, 475-492, doi:10.1111/joim.13032 (2020).

2 Wolfe, R. M. & Ang, D. C. Biologic Therapies for Autoimmune and Connective Tissue Diseases. *Immunology and allergy clinics of North America* **37**, 283-299, doi:10.1016/j.iac.2017.01.005 (2017).

3 Qin, B. *et al.* Epidemiology of primary Sjögren's syndrome: a systematic review and meta-analysis. *Annals of the rheumatic diseases* **74**, 1983-1989, doi:10.1136/annrheumdis-2014-205375 (2015).

4 Kvarnstrom, M., Ottosson, V., Nordmark, B. & Wahren-Herlenius, M. Incident cases of primary Sjogren's syndrome during a 5-year period in Stockholm County: a descriptive study of the patients and their characteristics. *Scandinavian Journal of Rheumatology* **44**, 135-142, doi:10.3109/03009742.2014.931457 (2015).

5 Brito-Zeron, P. *et al.* How immunological profile drives clinical phenotype of primary Sjogren's syndrome at diagnosis: analysis of 10,500 patients (Sjogren Big Data Project). *Clinical and experimental rheumatology* **36 Suppl 112**, 102-112 (2018).

6 Brito-Zerón, P. *et al.* Epidemiological profile and north-south gradient driving baseline systemic involvement of primary Sjögren's syndrome. *Rheumatology (Oxford, England)* **59**, 2350-2359, doi:10.1093/rheumatology/kez578 (2020).

7 Brkic, Z. *et al.* Prevalence of interferon type I signature in CD14 monocytes of patients with Sjogren's syndrome and association with disease activity and BAFF gene expression. *Annals of the rheumatic diseases* **72**, 728-735, doi:10.1136/annrheumdis-2012-201381 (2013).

8 Rose, T. *et al.* SIGLEC1 is a biomarker of disease activity and indicates extraglandular manifestation in primary Sjogren's syndrome. *RMD open* **2**, e000292, doi:10.1136/rmdopen-2016-000292 (2016).

9 Brito-Zeron, P. *et al.* Systemic activity and mortality in primary Sjogren syndrome: predicting survival using the EULAR-SS Disease Activity Index (ESSDAI) in 1045 patients. *Annals of the rheumatic diseases* **75**, doi:10.1136/annrheumdis-2014-206418 (2016).

10 Singh, A. G., Singh, S. & Matteson, E. L. Rate, risk factors and causes of mortality in patients with Sjögren's syndrome: a systematic review and meta-analysis of cohort studies. *Rheumatology (Oxford, England)* **55**, 450-460, doi:10.1093/rheumatology/kev354 (2016).

11 Theander, E., Manthorpe, R. & Jacobsson, L. T. Mortality and causes of death in primary Sjögren's syndrome: a prospective cohort study. *Arthritis and rheumatism* **50**, 1262-1269, doi:10.1002/art.20176 (2004).

12 Vasaitis, L. *et al.* Comparison of patients with and without pre-existing lymphoma at diagnosis of primary Sjögren's syndrome. *Scand J Rheumatol* **48**, 207-212, doi:10.1080/03009742.2018.1523456 (2019).

13 Vasaitis, L. *et al.* Population-based study of patients with primary Sjögren's syndrome and lymphoma: lymphoma subtypes, clinical characteristics, and gender differences. *Scand J Rheumatol* **49**, 225-232, doi:10.1080/03009742.2019.1696403 (2020).

14 Theander, E. *et al.* Lymphoid organisation in labial salivary gland biopsies is a possible predictor for the development of malignant lymphoma in primary Sjögren's syndrome. *Annals of the rheumatic diseases* **70**, 1363-1368, doi:10.1136/ard.2010.144782 (2011).

15 Leverenz, D. L. & St Clair, E. W. Recent advances in the search for a targeted immunomodulatory therapy for primary Sjögren's syndrome. *F1000Research* **8**, doi:10.12688/f1000research.19842.1 (2019).

16 Meiners, P. M. *et al.* Abatacept treatment reduces disease activity in early primary Sjögren's syndrome (open-label proof of concept ASAP study). *Annals of the rheumatic diseases* **73**, 1393-1396, doi:10.1136/annrheumdis-2013-204653 (2014).

17 St Clair, E. W. *et al.* Rituximab therapy for primary Sjögren's syndrome: an open-label clinical trial and mechanistic analysis. *Arthritis and rheumatism* **65**, 1097-1106, doi:10.1002/art.37850 (2013).

18 Devauchelle-Pensec, V. *et al.* Treatment of primary Sjögren syndrome with rituximab: a randomized trial. *Annals of internal medicine* **160**, 233-242, doi:10.7326/m13-1085 (2014).

19 Bowman, S. J. *et al.* Randomized Controlled Trial of Rituximab and Cost-Effectiveness Analysis in Treating Fatigue and Oral Dryness in Primary Sjogren's Syndrome. *Arthritis & rheumatology (Hoboken, N.J.)* **69**, 1440-1450, doi:10.1002/art.40093 (2017).

20 Mariette, X. *et al.* Efficacy and safety of belimumab in primary Sjögren's syndrome: results of the BELISS open-label phase II study. *Annals of the rheumatic diseases* **74**, 526-531, doi:10.1136/annrheumdis-2013-203991 (2015).

21 Dörner, T. *et al.* Treatment of primary Sjögren's syndrome with ianalumab (VAY736) targeting B cells by BAFF receptor blockade coupled with enhanced, antibody-dependent cellular cytotoxicity. *Annals of the rheumatic diseases* **78**, 641-647, doi:10.1136/annrheumdis-2018-214720 (2019).

22 Pontarini, E. *et al.* Treatment with belimumab restores B cell subsets and their expression of B cell activating factor receptor in patients with primary Sjogren's syndrome. *Rheumatology (Oxford, England)* **54**, 1429-1434, doi:10.1093/rheumatology/kev005 (2015).

23 Seror, R. *et al.* Low numbers of blood and salivary natural killer cells are associated with a better response to belimumab in primary Sjogren's syndrome: results of the BELISS study. *Arthritis research & therapy* **17**, 241, doi:10.1186/s13075-015-0750-y (2015).

24 Anquetil, C. *et al.* Is early-onset primary Sjögren's syndrome a worse prognosis form of the disease? *Rheumatology (Oxford, England)* **58**, 1163-1167, doi:10.1093/rheumatology/key392 (2019).

25 Baldini, C., Ferro, F., Luciano, N., Bombardieri, S. & Grossi, E. Artificial neural networks help to identify disease subsets and to predict lymphoma in primary Sjögren's syndrome. *Clinical and experimental rheumatology* **36 Suppl 112**, 137-144 (2018).

26 Theander, E. *et al.* Prediction of Sjogren's Syndrome Years Before Diagnosis and Identification of Patients With Early Onset and Severe Disease Course by Autoantibody Profiling. *Arthritis & rheumatology (Hoboken, N.J.)* **67**, 2427-2436, doi:10.1002/art.39214 (2015).

27 Brito-Zerón, P. *et al.* Influence of geolocation and ethnicity on the phenotypic expression of primary Sjögren's syndrome at diagnosis in 8310 patients: a cross-sectional study from the Big Data Sjögren Project Consortium. *Annals of the rheumatic diseases* **76**, 1042-1050, doi:10.1136/annrheumdis-2016-209952 (2017).

28 Shiboski, C. H. *et al.* 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjogren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis & rheumatology (Hoboken, N.J.)* **69**, 35-45, doi:10.1002/art.39859 (2017).

29 Robbins, A. *et al.* Diagnostic Utility of Separate Anti-Ro60 and Anti-Ro52/TRIM21 Antibody Detection in Autoimmune Diseases. *Front Immunol* **10**, 444, doi:10.3389/fimmu.2019.00444 (2019).

30 Baer, A. N. *et al.* The SSB-positive/SSA-negative antibody profile is not associated with key phenotypic features of Sjogren's syndrome. *Annals of the rheumatic diseases* **74**, 1557-1561, doi:10.1136/annrheumdis-2014-206683 (2015).

31 Acar-Denizli, N. *et al.* Systemic phenotype related to primary Sjögren's syndrome in 279 patients carrying isolated anti-La/SSB antibodies. *Clinical and experimental rheumatology* **38 Suppl 126**, 85-94 (2020).

32 Quartuccio, L. *et al.* Anti-SSA/SSB-negative Sjogren’s syndrome shows a lower prevalence of lymphoproliferative manifestations, and a lower risk of lymphoma evolution. *Autoimmun Rev* **14**, doi:10.1016/j.autrev.2015.07.002 (2015).

33 Retamozo, S. *et al.* Systemic manifestations of primary Sjögren's syndrome out of the ESSDAI classification: prevalence and clinical relevance in a large international, multi-ethnic cohort of patients. *Clinical and experimental rheumatology* **37 Suppl 118**, 97-106 (2019).

34 Hernández-Molina, G. *et al.* Usefulness of IgA Anti-α-fodrin Antibodies in Combination with Rheumatoid Factor and/or Antinuclear Antibodies as Substitute Immunological Criterion in Sjögren Syndrome with Negative Anti-SSA/SSB Antibodies. *J Rheumatol* **43**, 1852-1857, doi:10.3899/jrheum.151315 (2016).

35 Bournia, V. K. & Vlachoyiannopoulos, P. G. Subgroups of Sjögren syndrome patients according to serological profiles. *Journal of autoimmunity* **39**, 15-26, doi:10.1016/j.jaut.2012.03.001 (2012).

36 Ter Borg, E. J. & Kelder, J. C. Is extra-glandular organ damage in primary Sjögren's syndrome related to the presence of systemic auto-antibodies and/or hypergammaglobulinemia? A long-term cohort study with 110 patients from the Netherlands. *International journal of rheumatic diseases* **20**, 875-881, doi:10.1111/1756-185x.13070 (2017).

37 Kimman, J., Bossuyt, X. & Blockmans, D. Prognostic value of cryoglobulins, protein electrophoresis, and serum immunoglobulins for lymphoma development in patients with Sjögren's syndrome. A retrospective cohort study. *Acta clinica Belgica* **73**, 169-181, doi:10.1080/17843286.2017.1373966 (2018).

38 Retamozo, S. *et al.* Cryoglobulinaemic vasculitis at diagnosis predicts mortality in primary Sjogren syndrome: analysis of 515 patients. *Rheumatology (Oxford, England)* **55**, doi:10.1093/rheumatology/kew194 (2016).

39 Baldini, C. *et al.* Overlap of ACA-positive systemic sclerosis and Sjögren's syndrome: a distinct clinical entity with mild organ involvement but at high risk of lymphoma. *Clinical and experimental rheumatology* **31**, 272-280 (2013).

40 Lee, K. E. *et al.* Anti-centromere antibody-positive Sjögren's syndrome: A distinct clinical subgroup? *International journal of rheumatic diseases* **18**, 776-782, doi:10.1111/1756-185x.12684 (2015).

41 Shiboski, C. H. *et al.* Natural History and Predictors of Progression to Sjögren's Syndrome Among Participants of the Sjögren's International Collaborative Clinical Alliance Registry. *Arthritis Care Res (Hoboken)* **70**, 284-294, doi:10.1002/acr.23264 (2018).

42 Kuo, C. F. *et al.* Familial Risk of Sjogren's Syndrome and Co-aggregation of Autoimmune Diseases in Affected Families: A Nationwide Population Study. *Arthritis & Rheumatology* **67**, 1904-1912, doi:10.1002/art.39127 (2015).

43 Lessard, C. J. *et al.* Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. *Nature genetics* **45**, 1284-1292, doi:10.1038/ng.2792 (2013).

44 Nordmark, G. *et al.* Additive effects of the major risk alleles of IRF5 and STAT4 in primary Sjogren's syndrome. *Genes and immunity* **10**, 68-76, doi:10.1038/gene.2008.94 (2009).

45 Nordmark, G. *et al.* Association of EBF1, FAM167A(C8orf13)-BLK and TNFSF4 gene variants with primary Sjogren's syndrome. *Genes and immunity* **12**, 100-109, doi:10.1038/gene.2010.44 (2011).

46 Nordmark, G. *et al.* Association of genes in the NF-κB pathway with antibody-positive primary Sjögren's syndrome. *Scand J Immunol* **78**, 447-454, doi:10.1111/sji.12101 (2013).

47 Sun, F. *et al.* Association studies of TNFSF4, TNFAIP3 and FAM167A-BLK polymorphisms with primary Sjogren's syndrome in Han Chinese. *Journal of human genetics* **58**, 475-479, doi:10.1038/jhg.2013.26 (2013).

48 Li, Y. Z. *et al.* A genome-wide association study in Han Chinese identifies a susceptibility locus for primary Sjogren's syndrome at 7q11.23. *Nature genetics* **45**, 1361-1365, doi:10.1038/ng.2779 (2013).

49 Harris, V. M., Scofield, R. H. & Sivils, K. L. Genetics in Sjögren's syndrome: where we are and where we go. *Clinical and experimental rheumatology* **37 Suppl 118**, 234-239 (2019).

50 Imgenberg-Kreuz, J., Rasmussen, A., Sivils, K. & Nordmark, G. Genetics and epigenetics in primary Sjogren's syndrome. *Rheumatology (Oxford, England)*, doi:10.1093/rheumatology/key330 (2019).

51 Zheng, J. F. *et al.* The GTF2I rs117026326 polymorphism is associated with anti-SSA-positive primary Sjogren's syndrome. *Rheumatology* **54**, 562-564, doi:10.1093/rheumatology/keu466 (2015).

52 Song, I. W. *et al.* Identification of susceptibility gene associated with female primary Sjögren's syndrome in Han Chinese by genome-wide association study. *Human genetics* **135**, 1287-1294, doi:10.1007/s00439-016-1716-0 (2016).

53 Ciccacci, C. *et al.* TNFAIP3 Gene Polymorphisms in Three Common Autoimmune Diseases: Systemic Lupus Erythematosus, Rheumatoid Arthritis, and Primary Sjogren Syndrome-Association with Disease Susceptibility and Clinical Phenotypes in Italian Patients. *Journal of immunology research* **2019**, 6728694, doi:10.1155/2019/6728694 (2019).

54 Nezos, A. *et al.* TNFAIP3 F127C Coding Variation in Greek Primary Sjogren's Syndrome Patients. *Journal of immunology research* **2018**, 6923213-6923213, doi:10.1155/2018/6923213 (2018).

55 Taylor, K. E. *et al.* Genome-Wide Association Analysis Reveals Genetic Heterogeneity of Sjogren's Syndrome According to Ancestry. *Arthritis & rheumatology (Hoboken, N.J.)* **69**, 1294-1305, doi:10.1002/art.40040 (2017).

56 Gregersen, P. K. *et al.* REL, encoding a member of the NF-[kappa]B family of transcription factors, is a newly defined risk locus for rheumatoid arthritis. *Nature genetics* **41**, 820-823, doi:http://www.nature.com/ng/journal/v41/n7/suppinfo/ng.395_S1.html (2009).

57 Hom, G. *et al.* Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *The New England journal of medicine* **358**, 900-909, doi:10.1056/NEJMoa0707865 (2008).

58 Gourh, P. *et al.* Association of the C8orf13-BLK region with systemic sclerosis in North-American and European populations. *Journal of autoimmunity* **34**, 155-162, doi:10.1016/j.jaut.2009.08.014 (2010).

59 Miller, F. W. *et al.* Genome-Wide Association Study of Dermatomyositis Reveals Genetic Overlap With Other Autoimmune Disorders. *Arthritis and rheumatism* **65**, 3239-3247, doi:10.1002/art.38137 (2013).

60 Yin, H. *et al.* Association of STAT4 and BLK, but not BANK1 or IRF5, with primary antiphospholipid syndrome. *Arthritis and rheumatism* **60**, 2468-2471, doi:10.1002/art.24701 (2009).

61 Onouchi, Y. *et al.* A genome-wide association study identifies three new risk loci for Kawasaki disease. *Nature genetics* **44**, 517-521, doi:10.1038/ng.2220 (2012).

62 Thalayasingam, N. *et al.* CD4+ and B Lymphocyte Expression Quantitative Traits at Rheumatoid Arthritis Risk Loci in Patients With Untreated Early Arthritis: Implications for Causal Gene Identification. *Arthritis & rheumatology (Hoboken, N.J.)* **70**, 361-370, doi:10.1002/art.40393 (2018).

63 Chused, T. M., Kassan, S. S., Opelz, G., Moutsopoulos, H. M. & Terasaki, P. I. Sjögren's syndrome association with HLA-Dw3. *The New England journal of medicine* **296**, 895-897, doi:10.1056/nejm197704212961602 (1977).

64 Whittingham, S., Mackay, I. R. & Tait, B. D. Autoantibodies to small nuclear ribonucleoproteins. A strong association between anti-SS-B(La), HLA-B8, and Sjögren's syndrome. *Australian and New Zealand journal of medicine* **13**, 565-570, doi:10.1111/j.1445-5994.1983.tb02604.x (1983).

65 Cruz-Tapias, P., Rojas-Villarraga, A., Maier-Moore, S. & Anaya, J. M. HLA and Sjögren's syndrome susceptibility. A meta-analysis of worldwide studies. *Autoimmun Rev* **11**, 281-287, doi:10.1016/j.autrev.2011.10.002 (2012).

66 Gestermann, N. *et al.* Methylation profile of the promoter region of IRF5 in primary Sjögren's syndrome. *European cytokine network* **23**, 166-172, doi:10.1684/ecn.2012.0316 (2012).

67 Miceli-Richard, C. *et al.* Overlap between differentially methylated DNA regions in blood B lymphocytes and genetic at-risk loci in primary Sjögren's syndrome. *Annals of the rheumatic diseases* **75**, 933-940, doi:10.1136/annrheumdis-2014-206998 (2016).

68 Arvaniti, P. *et al.* Linking genetic variation with epigenetic profiles in Sjögren's syndrome. *Clinical immunology (Orlando, Fla.)* **210**, 108314, doi:10.1016/j.clim.2019.108314 (2020).

69 Imgenberg-Kreuz, J. *et al.* Genome-wide DNA methylation analysis in multiple tissues in primary Sjogren's syndrome reveals regulatory effects at interferon-induced genes. *Annals of the rheumatic diseases* **75**, 2029-2036, doi:10.1136/annrheumdis-2015-208659 (2016).

70 Ramirez Sepulveda, J. I., Kvarnstrom, M., Brauner, S., Baldini, C. & Wahren-Herlenius, M. Difference in clinical presentation between women and men in incident primary Sjogren's syndrome. *Biology of sex differences* **8**, 16, doi:10.1186/s13293-017-0137-7 (2017).

71 Flores-Chávez, A. *et al.* Severe, life-threatening phenotype of primary Sjögren's syndrome: clinical characterisation and outcomes in 1580 patients (GEAS-SS Registry). *Clinical and experimental rheumatology* **36 Suppl 112**, 121-129 (2018).

72 Langefeld, C. D. *et al.* Transancestral mapping and genetic load in systemic lupus erythematosus. *Nature communications* **8**, 16021, doi:10.1038/ncomms16021 (2017).

73 Scofield, R. H. *et al.* American Indians Have a Higher Risk of Sjögren's Syndrome and More Disease Activity Than European Americans and African Americans. *Arthritis Care Res (Hoboken)* **72**, 1049-1056, doi:10.1002/acr.24003 (2020).

74 Pontes, L. T., Camilo, D. T., De Bortoli, M. R., Santos, R. S. S. & Luchi, W. M. New-onset lupus nephritis after male-to-female sex reassignment surgery. *Lupus* **27**, 2166-2169, doi:10.1177/0961203318800571 (2018).

75 Haring, R. *et al.* Age-specific reference ranges for serum testosterone and androstenedione concentrations in women measured by liquid chromatography-tandem mass spectrometry. *The Journal of clinical endocrinology and metabolism* **97**, 408-415, doi:10.1210/jc.2011-2134 (2012).

76 Gubbels Bupp, M. R. & Jorgensen, T. N. Androgen-Induced Immunosuppression. *Front Immunol* **9**, 794, doi:10.3389/fimmu.2018.00794 (2018).

77 Aguilar-Pimentel, J. A. *et al.* Increased estrogen to androgen ratio enhances immunoglobulin levels and impairs B cell function in male mice. *Scientific reports* **10**, 18334, doi:10.1038/s41598-020-75059-9 (2020).

78 Erlandsson, M. C., Jonsson, C. A., Islander, U., Ohlsson, C. & Carlsten, H. Oestrogen receptor specificity in oestradiol-mediated effects on B lymphopoiesis and immunoglobulin production in male mice. *Immunology* **108**, 346-351, doi:10.1046/j.1365-2567.2003.01599.x (2003).

79 Ercan, A. *et al.* Estrogens regulate glycosylation of IgG in women and men. *JCI insight* **2**, e89703-e89703, doi:10.1172/jci.insight.89703 (2017).

80 Engdahl, C. *et al.* Estrogen induces St6gal1 expression and increases IgG sialylation in mice and patients with rheumatoid arthritis: a potential explanation for the increased risk of rheumatoid arthritis in postmenopausal women. *Arthritis research & therapy* **20**, 84, doi:10.1186/s13075-018-1586-z (2018).

81 Ainola, M. *et al.* Activation of plasmacytoid dendritic cells by apoptotic particles - mechanism for the loss of immunological tolerance in Sjögren's syndrome. *Clinical and experimental immunology* **191**, 301-310, doi:10.1111/cei.13077 (2018).

82 Jones, J. M. & Jørgensen, T. N. Androgen-Mediated Anti-inflammatory Cellular Processes as Therapeutic Targets in Lupus. *Front Immunol* **11**, 1271, doi:10.3389/fimmu.2020.01271 (2020).

83 Seminog, O. O., Seminog, A. B., Yeates, D. & Goldacre, M. J. Associations between Klinefelter's syndrome and autoimmune diseases: English national record linkage studies. *Autoimmunity* **48**, 125-128, doi:10.3109/08916934.2014.968918 (2015).

84 Harris, V. M. *et al.* Klinefelter's syndrome (47,XXY) is in excess among men with Sjögren's syndrome. *Clinical immunology (Orlando, Fla.)* **168**, 25-29, doi:10.1016/j.clim.2016.04.002 (2016).

85 Jørgensen, K. T. *et al.* Autoimmune diseases in women with Turner's syndrome. *Arthritis and rheumatism* **62**, 658-666, doi:10.1002/art.27270 (2010).

86 De Sanctis, V. & Khater, D. Autoimmune diseases in Turner syndrome: an overview. *Acta bio-medica : Atenei Parmensis* **90**, 341-344, doi:10.23750/abm.v90i3.8737 (2019).

87 Liu, K. *et al.* X Chromosome Dose and Sex Bias in Autoimmune Diseases: Increased Prevalence of 47,XXX in Systemic Lupus Erythematosus and Sjögren's Syndrome. *Arthritis & rheumatology (Hoboken, N.J.)* **68**, 1290-1300, doi:10.1002/art.39560 (2016).

88 Sharma, R. *et al.* Rare X Chromosome Abnormalities in Systemic Lupus Erythematosus and Sjogren's Syndrome. *Arthritis & rheumatology (Hoboken, N.J.)* **69**, 2187-2192, doi:10.1002/art.40207 (2017).

89 Zhu, Z. *et al.* Discovery of a novel genetic susceptibility locus on X chromosome for systemic lupus erythematosus. *Arthritis research & therapy* **17**, 349, doi:10.1186/s13075-015-0857-1 (2015).

90 Shen, N. *et al.* Sex-specific association of X-linked Toll-like receptor 7 (TLR7) with male systemic lupus erythematosus. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 15838-15843, doi:10.1073/pnas.1001337107 (2010).

91 Li, S. *et al.* Association study between X-linked susceptibility genes and clinical features in Chinese female patients with systemic lupus erythematosus. *Autoimmunity* **52**, 289-293, doi:10.1080/08916934.2019.1688792 (2019).

92 Mougeot, J. L., Noll, B. D. & Bahrani Mougeot, F. K. Sjögren's syndrome X-chromosome dose effect: An epigenetic perspective. *Oral diseases* **25**, 372-384, doi:10.1111/odi.12825 (2019).

93 Ramirez Sepulveda, J. I. *et al.* Long-term follow-up in primary Sjogren's syndrome reveals differences in clinical presentation between female and male patients. *Biology of sex differences* **8**, 25, doi:10.1186/s13293-017-0146-6 (2017).

94 Stein, C. M. *et al.* Increased prevalence of renal disease in systemic lupus erythematosus families with affected male relatives. *Arthritis and rheumatism* **46**, 428-435, doi:10.1002/art.10105 (2002).

95 Chen, L. *et al.* Genome-wide assessment of genetic risk for systemic lupus erythematosus and disease severity. *Human molecular genetics* **29**, 1745-1756, doi:10.1093/hmg/ddaa030 (2020).

96 Hughes, T. *et al.* Analysis of autosomal genes reveals gene–sex interactions and higher total genetic risk in men with systemic lupus erythematosus. *Annals of the rheumatic diseases* **71**, 694-699, doi:10.1136/annrheumdis-2011-200385 (2012).

97 Kovats, S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cellular immunology* **294**, 63-69, doi:10.1016/j.cellimm.2015.01.018 (2015).

98 de Weerd, N. A. & Nguyen, T. The interferons and their receptors--distribution and regulation. *Immunology and cell biology* **90**, 483-491, doi:10.1038/icb.2012.9 (2012).

99 Barrat, F. J., Crow, M. K. & Ivashkiv, L. B. Interferon target-gene expression and epigenomic signatures in health and disease. *Nature immunology* **20**, 1574-1583, doi:10.1038/s41590-019-0466-2 (2019).

100 Zhang, S. Y. *et al.* Inborn errors of interferon (IFN)-mediated immunity in humans: insights into the respective roles of IFN-alpha/beta, IFN-gamma, and IFN-lambda in host defense. *Immunological reviews* **226**, 29-40, doi:10.1111/j.1600-065X.2008.00698.x (2008).

101 Oke, V. *et al.* High levels of circulating interferons type I, type II and type III associate with distinct clinical features of active systemic lupus erythematosus. *Arthritis research & therapy* **21**, 107, doi:10.1186/s13075-019-1878-y (2019).

102 Thorlacius, G. E., Wahren-Herlenius, M. & Ronnblom, L. An update on the role of type I interferons in systemic lupus erythematosus and Sjogren's syndrome. *Curr Opin Rheumatol* **30**, 471-481, doi:10.1097/bor.0000000000000524 (2018).

103 Crow, Y. J. *et al.* Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutières syndrome and mimic congenital viral brain infection. *Nature genetics* **38**, 910-916, doi:10.1038/ng1842 (2006).

104 Cheng, M. H. & Anderson, M. S. in *Annual Review of Immunology, Vol 30* Vol. 30 *Annual Review of Immunology* (ed W. E. Paul) 393-427 (Annual Reviews, 2012).

105 Rodero, M. P. & Crow, Y. J. Type I interferon-mediated monogenic autoinflammation: The type I interferonopathies, a conceptual overview. *The Journal of experimental medicine* **213**, 2527-2538, doi:10.1084/jem.20161596 (2016).

106 Nordmark, G., Eloranta, M. L. & Ronnblom, L. Primary Sjogren's syndrome and the type I interferon system. *Current pharmaceutical biotechnology* **13**, 2054-2062 (2012).

107 Imgenberg-Kreuz, J. *et al.* Transcription profiling of peripheral B cells in antibody-positive primary Sjögren's syndrome reveals upregulated expression of CX3CR1 and a type I and type II interferon signature. *Scand J Immunol* **87**, e12662, doi:10.1111/sji.12662 (2018).

108 Hillen, M. R. *et al.* Plasmacytoid DCs From Patients With Sjögren's Syndrome Are Transcriptionally Primed for Enhanced Pro-inflammatory Cytokine Production. *Front Immunol* **10**, 2096, doi:10.3389/fimmu.2019.02096 (2019).

109 Apostolou, E. *et al.* Expression of type III interferons (IFNλs) and their receptor in Sjögren's syndrome. *Clinical & Experimental Immunology* **186**, 304-312, doi:10.1111/cei.12865 (2016).

110 Hall, J. C. *et al.* Molecular Subsetting of Interferon Pathways in Sjogren's Syndrome. *Arthritis & rheumatology (Hoboken, N.J.)* **67**, 2437-2446, doi:10.1002/art.39204 (2015).

111 Oke, V. *et al.* IFN-lambda1 with Th17 axis cytokines and IFN-alpha define different subsets in systemic lupus erythematosus (SLE). *Arthritis research & therapy* **19**, 139, doi:10.1186/s13075-017-1344-7 (2017).

112 Bodewes, I. L. A., Björk, A., Versnel, M. A. & Wahren-Herlenius, M. Innate immunity and interferons in the pathogenesis of Sjögren's syndrome. *Rheumatology (Oxford, England)*, doi:10.1093/rheumatology/key360 (2019).

113 Mofors, J. *et al.* Concomitant Ro/SSA and La/SSB antibodies are biomarkers for the risk of venous thromboembolism and cerebral infarction in primary Sjogren's syndrome. *Journal of internal medicine* **286**, 458-468, doi:10.1111/joim.12941 (2019).

114 Groves, A. M., Allan, L. D. & Rosenthal, E. Outcome of isolated congenital complete heart block diagnosed in utero. *Heart (British Cardiac Society)* **75**, 190-194, doi:10.1136/hrt.75.2.190 (1996).

115 Levesque, K. *et al.* Description of 214 cases of autoimmune congenital heart block: Results of the French neonatal lupus syndrome. *Autoimmun Rev* **14**, 1154-1160, doi:10.1016/j.autrev.2015.08.005 (2015).

116 Ho, A. *et al.* Isolated Complete Heart Block in the Fetus. *The American journal of cardiology* **116**, 142-147, doi:10.1016/j.amjcard.2015.03.051 (2015).

117 Skog, A., Wahren-Herlenius, M., Sundström, B., Bremme, K. & Sonesson, S. E. Outcome and growth of infants fetally exposed to heart block-associated maternal anti-Ro52/SSA autoantibodies. *Pediatrics* **121**, e803-809, doi:10.1542/peds.2007-1659 (2008).

118 Skog, A., Tingstrom, J., Salomonsson, S., Sonesson, S. E. & Wahren-Herlenius, M. Neurodevelopment in children with and without congenital heart block born to anti-Ro/SSA-positive mothers. *Acta paediatrica (Oslo, Norway : 1992)* **102**, 40-46, doi:10.1111/apa.12049 (2013).

119 Salomonsson, S. *et al.* A population-based investigation of the autoantibody profile in mothers of children with atrioventricular block. *Scand J Immunol* **74**, 511-517, doi:10.1111/j.1365-3083.2011.02610.x (2011).

120 Brucato, A. *et al.* Risk of congenital complete heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis: A prospective study of 100 women. *Arthritis & Rheumatism* **44**, 1832-1835, doi:10.1002/1529-0131(200108)44:8<1832::aid-art320>3.0.co;2-c (2001).

121 Ambrosi, A. *et al.* Development of heart block in children of SSA/SSB-autoantibody-positive women is associated with maternal age and displays a season-of-birth pattern.

Annals of the rheumatic diseases **71**, 334-340, doi:10.1136/annrheumdis-2011-200207 (2012).

122 Brito-Zeron, P., Izmirly, P. M., Ramos-Casals, M., Buyon, J. P. & Khamashta, M. A. The clinical spectrum of autoimmune congenital heart block. *Nature reviews. Rheumatology* **11**, 301-312, doi:10.1038/nrrheum.2015.29 (2015).

123 Sonesson, S. E., Ambrosi, A. & Wahren-Herlenius, M. Benefits of fetal echocardiographic surveillance in pregnancies at risk of congenital heart block: single-center study of 212 anti-Ro52-positive pregnancies. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* **54**, 87-95, doi:10.1002/uog.20214 (2019).

124 Sonesson, S.-E. & Wahren-Herlenius, M. Surveillance of congenital heart block in highly specialised care. *The Lancet Rheumatology* **2**, e203-e204, doi:10.1016/S2665-9913(20)30028-X (2020).

125 Izmirly, P. M. *et al.* Maternal use of hydroxychloroquine is associated with a reduced risk of recurrent anti-SSA/Ro-antibody-associated cardiac manifestations of neonatal lupus. *Circulation* **126**, 76-82, doi:10.1161/circulationaha.111.089268 (2012).

126 Izmirly, P. *et al.* Hydroxychloroquine to Prevent Recurrent Congenital Heart Block in Fetuses of Anti-SSA/Ro-Positive Mothers. *Journal of the American College of Cardiology* **76**, 292-302, doi:10.1016/j.jacc.2020.05.045 (2020).

127 Salomonsson, S. *et al.* A serologic marker for fetal risk of congenital heart block. *Arthritis & Rheumatism* **46**, 1233-1241, doi:10.1002/art.10232 (2002).

128 Jaeggi, E., Laskin, C., Hamilton, R., Kingdom, J. & Silverman, E. The importance of the level of maternal anti-Ro/SSA antibodies as a prognostic marker of the development of cardiac neonatal lupus erythematosus a prospective study of 186 antibody-exposed fetuses and infants. *Journal of the American College of Cardiology* **55**, 2778-2784, doi:10.1016/j.jacc.2010.02.042 (2010).

129 Meisgen, S. *et al.* Environmental and lifestyle factors influencing risk of congenital heart block during pregnancy in anti-Ro/SSA-positive women. *RMD open* **3**, e000520, doi:10.1136/rmdopen-2017-000520 (2017).

130 Clancy, R. M. *et al.* Identification of candidate loci at 6p21 and 21q22 in a genome-wide association study of cardiac manifestations of neonatal lupus. *Arthritis and rheumatism* **62**, 3415-3424, doi:10.1002/art.27658 (2010).

131 Kyriakidis, N. C. *et al.* European families reveal MHC class I and II associations with autoimmune-mediated congenital heart block. *Annals of the rheumatic diseases* **77**, 1381-1382, doi:10.1136/annrheumdis-2018-212953 (2018).

132 Ainsworth, H. C. *et al.* Association of Natural Killer Cell Ligand Polymorphism HLA-C Asn80Lys With the Development of Anti-SSA/Ro-Associated Congenital Heart Block. *Arthritis & rheumatology (Hoboken, N.J.)* **69**, 2170-2174, doi:10.1002/art.40228 (2017).

133 Meisgen, S. *et al.* The HLA locus contains novel foetal susceptibility alleles for congenital heart block with significant paternal influence. *Journal of internal medicine* **275**, 640-651, doi:10.1111/joim.12179 (2014).

134 Båve, U. *et al.* Activation of the type I interferon system in primary Sjogren's syndrome: a possible etiopathogenic mechanism. *Arthritis and rheumatism* **52**, 1185-1195, doi:10.1002/art.20998 (2005).

135 Fritz, M., Vats, K. & Goyal, R. K. Neonatal lupus and IUGR following alpha-interferon therapy during pregnancy. *Journal of perinatology : official journal of the California Perinatal Association* **25**, 552-554, doi:10.1038/sj.jp.7211342 (2005).

136 Lisney, A. R. *et al.* High maternal expression of SIGLEC1 on monocytes as a surrogate marker of a type I interferon signature is a risk factor for the development of autoimmune congenital heart block. *Annals of the rheumatic diseases* **76**, 1476-1480, doi:10.1136/annrheumdis-2016-210927 (2017).

137 Clancy, R. M. *et al.* Siglec-1 Macrophages and the Contribution of IFN to the Development of Autoimmune Congenital Heart Block. *Journal of immunology (Baltimore, Md. : 1950)* **202**, 48-55, doi:10.4049/jimmunol.1800357 (2019).

138 Clancy, R. M. *et al.* Cardiac fibroblast transcriptome analyses support a role for interferogenic, profibrotic, and inflammatory genes in anti-SSA/Ro-associated congenital heart block. *American journal of physiology. Heart and circulatory physiology* **313**, H631-h640, doi:10.1152/ajpheart.00256.2017 (2017).

139 Suryawanshi, H. *et al.* Cell atlas of the foetal human heart and implications for autoimmune-mediated congenital heart block. *Cardiovascular research* **116**, 1446-1457, doi:10.1093/cvr/cvz257 (2020).

140 Eriksson, D. *et al.* Extended exome sequencing identifies BACH2 as a novel major risk locus for Addison's disease. *Journal of internal medicine* **280**, 595-608, doi:10.1111/joim.12569 (2016).

141 Skog, A., Lagnefeldt, L., Conner, P., Wahren-Herlenius, M. & Sonesson, S. E. Outcome in 212 anti-Ro/SSA-positive pregnancies and population-based incidence of congenital heart block. *Acta obstetricia et gynecologica Scandinavica* **95**, 98-105, doi:10.1111/aogs.12785 (2016).

142 Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: a tool for genome-wide complex trait analysis. *American journal of human genetics* **88**, 76-82, doi:10.1016/j.ajhg.2010.11.011 (2011).

143 Feng, X. *et al.* Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. *Arthritis and rheumatism* **54**, 2951-2962, doi:10.1002/art.22044 (2006).

144 Björk, A. *et al.* Protein and DNA methylation-based scores as surrogate markers for interferon system activation in patients with primary Sjögren's syndrome. *RMD open* **6**, doi:10.1136/rmdopen-2019-000995 (2020).

145 Cederblad, B. *et al.* Patients with systemic lupus erythematosus have reduced numbers of circulating natural interferon-alpha- producing cells. *Journal of autoimmunity* **11**, 465-470, doi:10.1006/jaut.1998.0215 (1998).

146 Rodero, M. P. *et al.* Detection of interferon alpha protein reveals differential levels and cellular sources in disease. *The Journal of experimental medicine* **214**, 1547-1555, doi:10.1084/jem.20161451 (2017).

147 Ricchiuti, V., Isenberg, D. & Muller, S. HLA association of anti-Ro60 and anti-Ro52 antibodies in Sjögren's syndrome. *Journal of autoimmunity* **7**, 611-621, doi:10.1006/jaut.1994.1045 (1994).

148 Miyagawa, S. *et al.* Polymorphisms of HLA class II genes and autoimmune responses to Ro/SS-A-La/SS-B among Japanese subjects. *Arthritis and rheumatism* **41**, 927-934, doi:10.1002/1529-0131(199805)41:5<927::aid-art21>3.0.co;2-r (1998).

149 Gottenberg, J. E. *et al.* In primary Sjogren's syndrome, HLA class II is associated exclusively with autoantibody production and spreading of the autoimmune response. *Arthritis and rheumatism* **48**, 2240-2245, doi:10.1002/art.11103 (2003).

150 Furukawa, H. *et al.* Association of increased frequencies of HLA-DPB1*05:01 with the presence of anti-Ro/SS-A and anti-La/SS-B antibodies in Japanese rheumatoid arthritis and systemic lupus erythematosus patients. *PloS one* **8**, e53910, doi:10.1371/journal.pone.0053910 (2013).

151 Menor Almagro, R. *et al.* Association of anti-Ro52, anti-Ro60 and anti-La antibodies with diagnostic, clinical and laboratory features in a referral hospital in Jerez, Spain. *Reumatologia clinica* **12**, 256-262, doi:10.1016/j.reuma.2015.10.010 (2016).

152 Murng, S. H. K. & Thomas, M. Clinical associations of the positive anti Ro52 without Ro60 autoantibodies: undifferentiated connective tissue diseases. *Journal of clinical pathology* **71**, 12-19, doi:10.1136/jclinpath-2015-203587 (2018).

153 Zampeli, E., Mavrommati, M., Moutsopoulos, H. M. & Skopouli, F. N. Anti-Ro52 and/or anti-Ro60 immune reactivity: autoantibody and disease associations. *Clinical and experimental rheumatology* **38 Suppl 126**, 134-141 (2020).

154 Fox, R. I. & Fox, C. M. Sjögren Syndrome: Why Do Clinical Trials Fail? *Rheumatic diseases clinics of North America* **42**, 519-530, doi:10.1016/j.rdc.2016.03.009 (2016).

155 Gottenberg, J. E. *et al.* Efficacy of rituximab in systemic manifestations of primary Sjogren's syndrome: results in 78 patients of the AutoImmune and Rituximab registry. *Annals of the rheumatic diseases* **72**, 1026-1031, doi:10.1136/annrheumdis-2012-202293 (2013).

156 Ramos-Casals, M. *et al.* EULAR recommendations for the management of Sjögren's syndrome with topical and systemic therapies. *Annals of the rheumatic diseases* **79**, 3, doi:10.1136/annrheumdis-2019-216114 (2020).

157 Winthrop, K. L. *et al.* Unmet need in rheumatology: reports from the Targeted Therapies meeting 2019. *Annals of the rheumatic diseases* **79**, 88-93, doi:10.1136/annrheumdis-2019-216151 (2020).

158 Oliva, M. *et al.* The impact of sex on gene expression across human tissues. *Science (New York, N.Y.)* **369**, eaba3066, doi:10.1126/science.aba3066 (2020).

159 Buniello, A. *et al.* The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res* **47**, D1005-d1012, doi:10.1093/nar/gky1120 (2019).

160 Oparina, N. Y. *et al.* PXK locus in systemic lupus erythematosus: fine mapping and functional analysis reveals novel susceptibility gene ABHD6. *Annals of the rheumatic diseases* **74**, e14, doi:10.1136/annrheumdis-2013-204909 (2015).

161 Vaughn, S. E. *et al.* Lupus risk variants in the PXK locus alter B-cell receptor internalization. *Frontiers in genetics* **5**, 450, doi:10.3389/fgene.2014.00450 (2014).

162 Darbani, B. The Expression and Polymorphism of Entry Machinery for COVID-19 in Human: Juxtaposing Population Groups, Gender, and Different Tissues. *International journal of environmental research and public health* **17**, doi:10.3390/ijerph17103433 (2020).

163 Liu, M. J. *et al.* ZIP8 regulates host defense through zinc-mediated inhibition of NF-κB. *Cell reports* **3**, 386-400, doi:10.1016/j.celrep.2013.01.009 (2013).

164 Nebert, D. W. & Liu, Z. SLC39A8 gene encoding a metal ion transporter: discovery and bench to bedside. *Human genomics* **13**, 51, doi:10.1186/s40246-019-0233-3 (2019).

165 Su, H., Na, N., Zhang, X. & Zhao, Y. The biological function and significance of CD74 in immune diseases. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]* **66**, 209-216, doi:10.1007/s00011-016-0995-1 (2017).

166 Zhou, Y. *et al.* CD74 Deficiency Mitigates Systemic Lupus Erythematosus-like Autoimmunity and Pathological Findings in Mice. *Journal of immunology (Baltimore, Md. : 1950)* **198**, 2568-2577, doi:10.4049/jimmunol.1600028 (2017).

167 Ramírez Sepúlveda, J. I. *et al.* Sex differences in clinical presentation of systemic lupus erythematosus. *Biology of sex differences* **10**, 60, doi:10.1186/s13293-019-0274-2 (2019).

168 Izumi, K. *et al.* ARCN1 Mutations Cause a Recognizable Craniofacial Syndrome Due to COPI-Mediated Transport Defects. *American journal of human genetics* **99**, 451-459, doi:10.1016/j.ajhg.2016.06.011 (2016).

169 Aktaş, T. *et al.* DHX9 suppresses RNA processing defects originating from the Alu invasion of the human genome. *Nature* **544**, 115-119, doi:10.1038/nature21715 (2017).

170 Hung, T. *et al.* The Ro60 autoantigen binds endogenous retroelements and regulates inflammatory gene expression. *Science (New York, N.Y.)* **350**, 455-459, doi:10.1126/science.aac7442 (2015).

171 Karolchik, D. *et al.* The UCSC Table Browser data retrieval tool. *Nucleic Acids Res* **32**, D493-496, doi:10.1093/nar/gkh103 (2004).

172 Salm, M. P. *et al.* The origin, global distribution, and functional impact of the human 8p23 inversion polymorphism. *Genome research* **22**, 1144-1153, doi:10.1101/gr.126037.111 (2012).

173 Namjou, B. *et al.* The effect of inversion at 8p23 on BLK association with lupus in Caucasian population. *PloS one* **9**, e115614, doi:10.1371/journal.pone.0115614 (2014).

174 GTEx-Consortium. The Genotype-Tissue Expression (GTEx) project. *Nature genetics* **45**, 580-585, doi:10.1038/ng.2653 (2013).

175 Aqrawi, L. A. *et al.* Clinical associations and expression pattern of the autoimmunity susceptibility factor DIORA-1 in patients with primary Sjögren's syndrome. *Annals of the rheumatic diseases* **77**, 1840-1842, doi:10.1136/annrheumdis-2018-213634 (2018).

176 Wright, P. E. & Dyson, H. J. Intrinsically disordered proteins in cellular signalling and regulation. *Nature Reviews Molecular Cell Biology* **16**, 18-29, doi:10.1038/nrm3920 (2015).

177 Uversky, V. N. *et al.* High-level intrinsic disorder explains the universality of CLIP binding to diverse MHC class II variants. *Cellular & Molecular Immunology* **15**, 76-78, doi:10.1038/cmi.2017.45 (2018).

178 Samuelson, E. M., Laird, R. M., Maue, A. C., Rochford, R. & Hayes, S. M. Blk haploinsufficiency impairs the development, but enhances the functional responses, of

MZ B cells. *Immunology and cell biology* **90**, 620-629, doi:10.1038/icb.2011.76 (2012).

179 Zhang, X., Mei, D., Zhang, L. & Wei, W. Src Family Protein Kinase Controls the Fate of B Cells in Autoimmune Diseases. *Inflammation*, doi:10.1007/s10753-020-01355-1 (2020).

180 Brucato, A. *et al.* Passively acquired anti-SSA/Ro antibodies are required for congenital heart block following ovodonation but maternal genes are not. *Arthritis and rheumatism* **62**, 3119-3121, doi:10.1002/art.27575 (2010).

181 Clancy, R. M. *et al.* Genetic association of cutaneous neonatal lupus with HLA class II and tumor necrosis factor alpha: implications for pathogenesis. *Arthritis and rheumatism* **50**, 2598-2603, doi:10.1002/art.20442 (2004).

182 Smith, C. O. *et al.* Cardiac metabolic effects of K(Na)1.2 channel deletion and evidence for its mitochondrial localization. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **32**, fj201800139R, doi:10.1096/fj.201800139R (2018).

183 Zhao, J. *et al.* Association of genetic variants in complement factor H and factor H-related genes with systemic lupus erythematosus susceptibility. *PLoS Genet* **7**, e1002079, doi:10.1371/journal.pgen.1002079 (2011).

184 Jönsen, A. *et al.* Mutations in genes encoding complement inhibitors CD46 and CFH affect the age at nephritis onset in patients with systemic lupus erythematosus. *Arthritis research & therapy* **13**, R206, doi:10.1186/ar3539 (2011).

185 Yang, X. *et al.* Genome-wide association study for serum complement C3 and C4 levels in healthy Chinese subjects. *PLoS Genet* **8**, e1002916, doi:10.1371/journal.pgen.1002916 (2012).

186 *Capture HiC Plotter (CHiCP)*, <https://www.chicp.org>

187 Munroe, M. E. *et al.* Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Annals of the rheumatic diseases* **75**, 2014-2021, doi:10.1136/annrheumdis-2015-208140 (2016).

188 Rönnblom, L. & Leonard, D. Interferon pathway in SLE: one key to unlocking the mystery of the disease. *Lupus science & medicine* **6**, e000270, doi:10.1136/lupus-2018-000270 (2019).

189 Eloranta, M. L. *et al.* Regulation of the interferon-alpha production induced by RNA-containing immune complexes in plasmacytoid dendritic cells. *Arthritis and rheumatism* **60**, 2418-2427, doi:10.1002/art.24686 (2009).

190 Ambrosi, A., Sonesson, S. E. & Wahren-Herlenius, M. Molecular mechanisms of congenital heart block. *Experimental cell research* **325**, 2-9, doi:10.1016/j.yexcr.2014.01.003 (2014).

191 Hansen, A., Daridon, C. & Dörner, T. What do we know about memory B cells in primary Sjögren's syndrome? *Autoimmun Rev* **9**, 600-603, doi:10.1016/j.autrev.2010.05.005 (2010).

192 Bodewes, I. L. A., Gottenberg, J. E., van Helden-Meeuwsen, C. G., Mariette, X. & Versnel, M. A. Hydroxychloroquine treatment downregulates systemic interferon activation in primary Sjögren's syndrome in the JOQUER randomized trial.

Rheumatology (Oxford, England) **59**, 107-111, doi:10.1093/rheumatology/kez242 (2020).

193 Salafia, C. M. & Parke, A. L. Placental pathology in systemic lupus erythematosus and phospholipid antibody syndrome. *Rheumatic diseases clinics of North America* **23**, 85-97 (1997).

194 Aaltonen, R., Heikkinen, T., Hakala, K., Laine, K. & Alanen, A. Transfer of proinflammatory cytokines across term placenta. *Obstetrics and gynecology* **106**, 802-807, doi:10.1097/01.AOG.0000178750.84837.ed (2005).

195 Tunks, R. D., Clowse, M. E., Miller, S. G., Brancazio, L. R. & Barker, P. C. Maternal autoantibody levels in congenital heart block and potential prophylaxis with antiinflammatory agents. *American journal of obstetrics and gynecology* **208**, 64.e61-67, doi:10.1016/j.ajog.2012.09.020 (2013).

196 Rival, C., Setiady, Y., Samy, E. T., Harakal, J. & Tung, K. S. The unique neonatal NK cells: a critical component required for neonatal autoimmune disease induction by maternal autoantibody. *Front Immunol* **5**, 242, doi:10.3389/fimmu.2014.00242 (2014).

197 Sips, M. *et al.* HLA-C levels impact natural killer cell subset distribution and function. *Hum Immunol* **77**, 1147-1153, doi:10.1016/j.humimm.2016.08.004 (2016).